STUDIES ON THE MICROBIAL DEGRADATION OF COMPLEX SUBSTRATES

By

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DEDICTION

In the name of Allah Most Gracious Most Merciful

This thesis is dedicated to the memory of my affectionate father. Also I dedicate this effort to my beloved Mother. A special dedication to those who inspired me to the higher ideals of life, my husband Mansur and my children Bssam, Nizar, Ans, Raghad and Feras.

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ABSTRACT

Microorganisms, mainly bacteria and fungi, are key agents involved in the breakdown and decomposition of plant and animal polymers in ecosystems. The aim of this research project was to study the mechanisms of degradation of four complex substrates; keratin, pectin, alginate and chitin. In this study, keratinophylic fungi were isolated from agricultural soil via a hair-baiting technique (HBT) using wool and hair as baits; additionally, keratinophylic species were isolated from contaminated feathers. The isolates from hair, wool and feathers were grown on solid media supported by keratin azure as a source of carbon and nitrogen. Keratinolytic activities were observed by the formation of a clearing zone in the medium. A study of keratinolytic assay in shaking culture was made by measuring the activity of keratinase (release keratin azure). In addition, scanning electron microscopy (SEM) studies were included in this study. Qualitative assays of pectin degradation, using apple pectin as a carbon source are reported. Pectin degradation in plates was detected using a solution of iodine-potassium iodide. Pectinase activity was determined in the supernatants by release of reducing sugars (galacturonic acid) using dinitrosalicylate reagent (DNS). Antimicrobial activities of pectin esterified potassium salt against some pathogens partically the bacteria which cause infection in wounds was determined by measuring inhibition zones around the wells. Alginolytic microorganisms were isolated from two fresh seaweeds, namely *Fucus* and *Laminaria.* The enzymatic activities were quantified by the formation of new unsaturated non-reducing ends and as reducing sugar (RS). The amount of reducing sugar formed was determined using 3, 5-dinitrosalicylic acid (DNS) methods. Crab shell chitin was hydrolysed by acid to produce colloidal chitin. Fungal and bacterial isolates were tested to determine chitinolytic properties in plates by measuring purple zones against yellow background. The supernatants derived from selected isolates were then used to determine chitinase activity by measuring reducing sugars (RS). RS calculated as glucose using Nelson and DNS methods. The fertilizer-potential of the substrates was determined by measuring nitrification and the oxidation of sulphur in soil amendment with the individual complex substrates. A variety of bacteria and fungal isolates were identified using molecular identification techniques. Finally, four enzymes were isolated and partially purified using ammonium sulphate in order to determine their molecular weight using SDS polyacrylamide gel electrophoresis (SDS-PAGE). In addition, liquid chromatography mass spectrometry (LC-MS/MS) has been used to identify three enzymes namely; keratinase, pectinases and chitinases.

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LITERATURE REVIEW

1.1. Introduction-the degradation and environmental impact of complex substrates

The random disposal of household, agricultural and industrial waste without regard for health and environmental effects has become a major issue across the world (Agwu and Kalu, 2012). Huge amounts of organic and inorganic compounds (domestic and industrial wastes) are released into the environment each year as a result of human activities. Subsequently, serious environmental problems have resulted. The contamination of soil, water and air is one of these problems which create a significant threat to the environment (Morelli *et al*., 2005). Several physical and chemical treatments have been proposed to deal with the waste such as incineration, chlorination, and ozonation. These methods have major drawbacks such as they are expensive and need complicated equipment and involve the use of large amounts of fuel (Mrayyan and Battikhi, 2005). Biological methods (biodegradation) provide an alternative to such treatment methods and biological approaches are increasingly being used to clean up of the environments.

1.2. Biodegradation of complex organic substrates

Biodegradation is a general term meaning the breakdown of chemical compounds by microorganisms in order to transform or degrade toxic compounds, or convert them to non-hazardous compounds which can enter natural biogeochemical cycles. Generally, biodegradation is a series of biochemical reactions that result in total breakdown of organic compounds; a process which is often referred to as "mineralization" (Allsopp *et al*., 2004). There are several factors which affect biodegradation such as nutrients, oxygen, pH value, composition, chemical and physical characteristics (Margesin and Schinner, 2001). The activities of microorganisms in these bio-treatments involve simple processes which degrade organic pollutants and transform inorganic pollutants (Gadd, 2008). Biodegradation is considered an effective option, because it provides less expensive and an environmentally friendly way of tackling those environmental problems which arise from current methods of waste treatment (Agwu and Kalu, 2012); it is reliable, simple and cheap and leads to a reduction in environmental pollutants (Margesin and Schinner, 2001).

1.2.1. Degradation of substrates by bacteria and fungi

Biodegradation is increasingly investigated in order to provide environmental friendly solutions to environmental problems. Biodegradation can be achieved by a wide range of microorganisms, notably bacteria and fungi. When the conditions are favourable for growth, bacteria and fungi are able to produce enzymes in order to obtain nutrients and energy. This process occurs through the degradation of complex substrates (Hernández and Hobbie, 2010).

A variety of bacteria are currently used in various stages of biotechnology, such as the production of valuable microbial products, biodegradation and the synthesis of organic compounds (Konopka *et al.*, 1996). Mycoremediation is one approach to bioremediaton in which fungi are used to remove chemical contaminants from the environment. Here, certain fungal enzymes and acids, which are naturally secreted, degrade hazardous chemicals and make them less, or non-toxic, compounds (Agwu and Kalu, 2012). Fungi are heterotrophic eukaryotes that utililize organic, energy-rich substrate when growing as saprophytes, parasites or symbionts. Due to these properties, fungi can obtain nutrients from both dead and living substrates (Osiewacz, 2002). Fungi must break down large molecules into smaller molecules before absorbing them through the cell walls and membranes. The breakdown of molecules is achieved by the secretion of extracellular enzymes that digest the food outside the fungal cell. Since fungi produce large amounts of a variety of enzymes they can readily utilize a range of different types of substrates as food sources (Gupta, 2004).

Many fungi can degrade complex and natural materials such as lignin, chitin and cellulose (Scott and Untereiner, 2004). In contrast to bacteria, fungi can extend the location of their biomass via hyphal growth. They can also grow under environmentally stressed conditions such as low nutrients, low water activity and low pH values where bacterial growth could be limited (Atagana, 2004). Fungi play a major role in the degradation of dead plant tissues (cellulose and lignin) and animal tissues such as keratin and chitin. A wide range of research has demonstrated the role of fungi as decomposers in the major cycles of nature (notably the C-cycle) and fungi are considered to be excellent degraders of complex organic substrates (Bennet *et al*., 2002) based on the following properties.

- Fungi can quickly branch throughout the substrate, digesting by secreting a series of extra-cellular enzymes.

- Fungi can tolerate high concentrations of toxic chemicals.

- Mycelial growth provides an advantage in aiding the colonization of complex insoluble substrates.

1.2.2. Biodegradation processes

Extracellular enzymes are generally produced by soil microbes which bring about the degradation of organic matter to release nutrients and carbon in simple forms (Fig 1.1). In addition, they help microorganisms obtain nutrients and energy from complex material in the environment (Allison and Vitousek, 2005). Extracellular enzymes are the principle agents that allow microbes to degrade insoluble biomass (from organic soil and waste) and convert them into soluble molecules which can then be assimilated. Several organic soil compounds can be degraded enzymatically such as proteins, chitin, cellulose, lipids and lignin. Extracellular enzymes might be linked with cell wall, plasma membrane or the periplasmic space. Extracellular enzymes which are released into the environment could be denatured or stabilized. A wide diversity of enzymes is present in soil as the result of the diversity of microorganisms, physical soil matrix and compounds in the soil. Some extracellular enzymes are released only in the presence of suitable substrates (inducible enzymes), while others are released regardless of substrate accessibility (constitutive enzymes), a fact which suggest the presence of a sensing mechanism used by microbes to recognize substrate accessibility in the environment (Wallenstein and Weintraub, 2008).

Extracellular enzymes such as glycosidases, esterases and peptidases are mostly hydrolases which cleave bonds (e.g. C-O and C-N bonds) located between monomers. Some extracellular enzymes are called oxidative enzymes because they can catalyze oxidative reactions by cleaving C-C and C-O bonds (Cunha *et al*., 2010).

1.3. Microbial degradation of proteins

Proteins are degraded using enzymes, a process termed proteolysis. Several proteins are degraded in the preliminary stages of animal tissue degradation, such as neuronal and epithelial tissue, while others are decomposed in the later stages such as collagen, epidermis, reticulin, muscle protein and keratin (Dent *et al*., 2004).

1.3.1. Keratins (KRTs) and keratinous substrates

Keratins are proteins characterized by high stability and low solubility due to –S–S– cross-linking between cysteine amino acids (Zoccola *et al*., 2009). Keratins are classified into two groups: hard keratin (hair, feather, nail, wool); and soft keratin (skin) depending on their

sulphur content (Gurav and Jadhav, 2012). The most important feature of keratin is its high sulphur content, due to the presence of sulphur- containing amino acids cystine, cysteine and methionine (Table 1.1). Keratin is considered a stable molecule because of the presence of disulfide bonds (Fig. 1.2); as a result, keratin is generally resistant to enzymatic degradation (Kim, 2003).

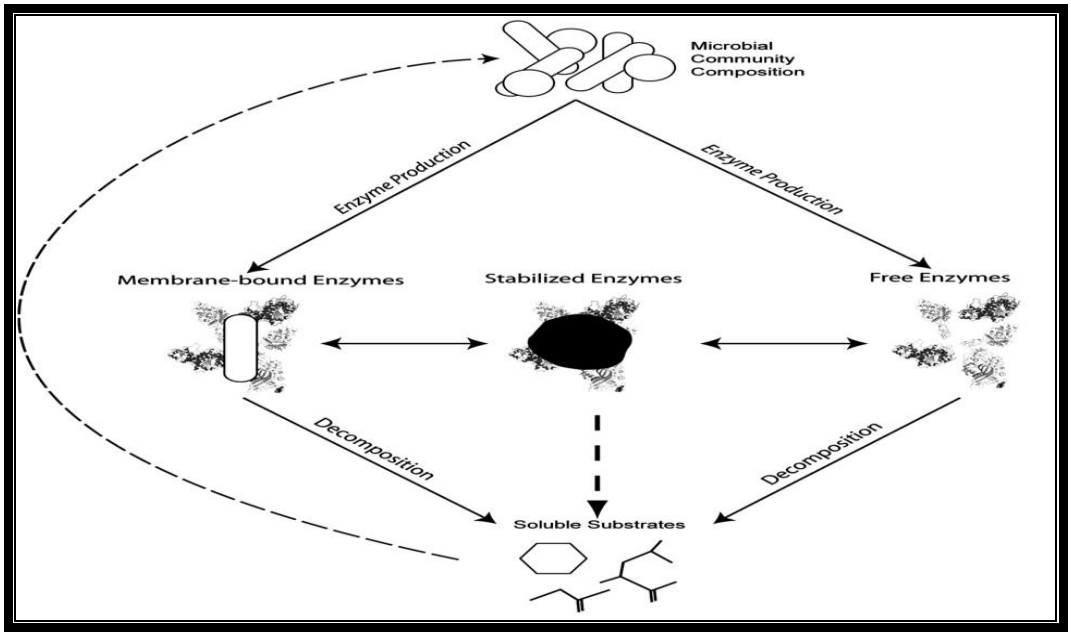


Figure 1.1: The relationship between microbial enzyme production, degradation, enzyme activity and stabilization of enzymes. Source (Wallenstein and Weintraub, 2008).

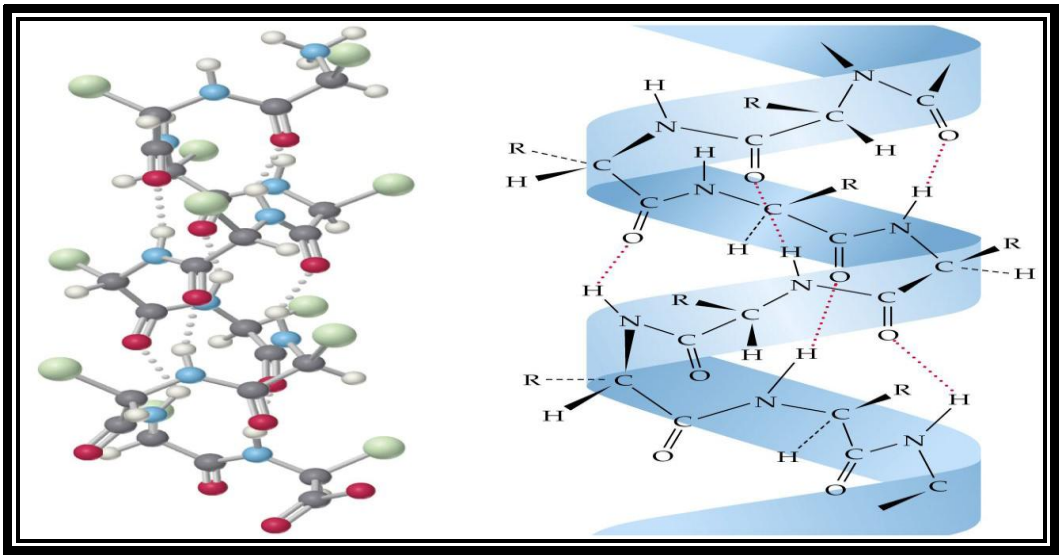


Figure 1.2: General structure of keratin; source (Jalendran and Dadvar, 2011).

Table 1.1: Percentage of nitrogen and sulphur in some keratinous materials; adapted from (Block, 1939).

Protein	Nitrogen %	Sulphur %
Human hair	15.4	5.0
Cow hair	15.3	3.7
Lamb wool	15.4	3.6
Camel wool	15.1	3.1
Cattle horn	16.1	2.6
Finger nails	14.9	3.8
Hen feather	15.5	2.3
Snake skin	15.2	2.2
Egg shell membrane	16.6	3.8

Incineration, recycling and land fill are physical solutions to treat a variety of keratinous waste. In several countries, most farm waste, feathers, hair and related materials are incinerated or discarded, causing energy waste and air pollution (Kuo *et al*., 2012). Recently, wastes, especially materials containing keratin, such as horns, hoof, feathers and wool have been recycled at high temperatures and pressure and milled to produce animal food (animal flour) which is commonly used as a protein supplement for feed mixtures for use by domestic animals (Tapia and Simões, 2008). Feather recycling is therefore used as an alternative protein supplement in feedstuffs or fertilizers (Riffel *et al*., 2007) . This chemical-based procedure leads to denaturation of sensitive and essential amino acids such as methionine, lysine and tryptophan during processing, which yields a product with poor digestibility and low nutritional value (Rodrigues Marcondes *et al*., 2008). Chlorination and ozonation are used as chemical treatments and such methods have drawbacks such as expense, the need for complex equipment and high fuel requirement (Mrayyan and Battikhi, 2005). Moreover, sodium sulphide, lime and other solvents used in the leather industry to hide some problems produce bad odours. These limitations have justified the search for an environmentally friendly method to deal with keratin-rich wastes without resulting in chemical pollution (Okoroma, 2012); these problems have necessitated a search for alternative ways to obtain proteins from waste, especially from bio-products of the poultry industry (Gurav and Jadhav, 2012).

1.3.2. The microbial keratinolysis process

When microorganisms locate a keratin-rich substrate they first utilize the non-keratinous components, such as lipids, and then begin to degrade keratin. Keratin molecules are used by microorganisms as a sources of carbon, nitrogen and sulphur (Marchisio, 2000). It is clear that the total degradation of keratinous material by specialized microorganisms can generate a chain of events. The keratinolysis process involves the breaking of disulfide bridges; i.e. called sulfitolysis and proteases alone cannot break the disulfide bonds and generate the complete hydrolysis of the keratin. Sulfitolysis is the key to keratinolysis (Błyskal, 2009). Several studies have considered the mechanism of keratinolysis but have failed to fully understand the process, although a two step reduction of disulfide bonds (sulfitolysis) and proteolysis using keratinolytic proteases is clearly involved (Liang *et al*., 2010).

1.3.2.1 Sulfitolysis

Keratinolytic microorganisms denature keratin by the process of sulphitolysis, which is absent in non-keratinolytic microorganisms. In this process, microbes initially release some sulphide responsible for the breakdown of disulphide bonds of cysteine present in keratin (Fig. 1.3). The proteolysis enzymes which are released then cleave this partially denatured protein (Sharma and Rajak, 2003). Microorganisms can use cysteine (free or combined) as a sulphur and nitrogen source and produce inorganic sulphur and other intermediate products. Microorganisms are not able to use whole cystine, and as a result, some sulphur returns to the medium in the oxidized form (sulphate and sulphite) (Onifade *et al*., 1998).

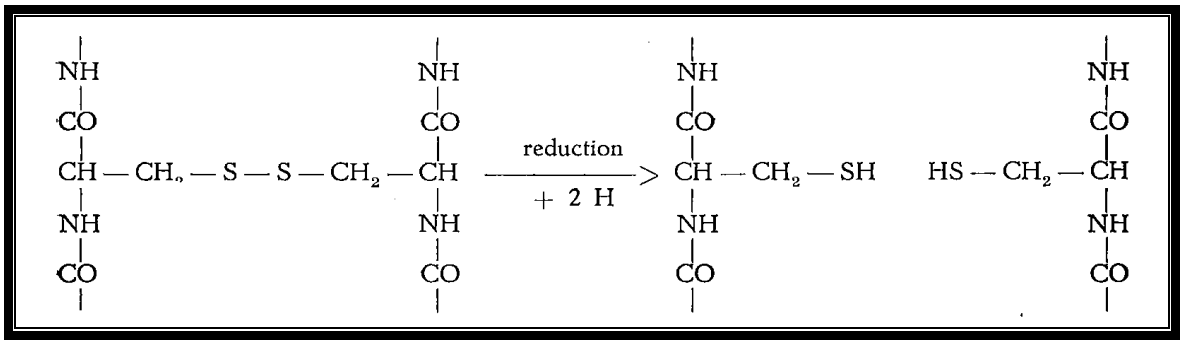


Figure 1.3: Breakdown of the disulphide bridge of keratin; source: (Mathison, 1964).

1.3.2.2. Proteolysis

Microbial degradation of keratin is achieved by specific proteases, i.e. keratinolytic enzymes (keratinases). Keratinase has been extracted from different microorganisms with high activity against keratin (Riffel *et al*., 2007). Microbial keratinases are mostly extracellular enzymes (Gupta and Ramnani, 2006). A few bacteria and fungi are capable of

degrading keratin waste efficiently due to their ability to secrete keratinase into the medium. Keratinase is also produced by insects but mostly by microorganisms, notably bacteria, saprophytic and parasitic fungi and actinomycetes (Tork *et al*., 2010). Fungal keratinases are produced by several species of fungi such as *Microsporum* and *Trichophyton* (Xie *et al*., 2010). Fungi are capable of producing enzymes tolerant to different pH values and are highly active at extreme pH values. As a result, these enzymes can be used in biotechnology and have potential wide application (Elíades *et al*., 2010).

1.3.3. The fungal keratinolysis process

Keratinous substrates such as hair are not made of keratin only but also contain some non-keratin components (e.g. lipid). Many fungi that grow on natural keratin substrates in nature do not actually use the keratin part but instead use the non-keratin fat-rich part of the substrate. Non-specialists species colonize first, followed by true keratinolytic fungi (Sharma and Rajak, 2003). There are two invasion paths of keratinous substrates used by fungi, namely:

1- **Surface erosion**

This is the gradual degradation which develops from the outside toward the centre; subsequently cuticle decay occurs to produce a wavy cortex.

2- **Radial penetration**

This is advanced invasion of the substrate achieved by more specialized hyphae. These hyphae penetrate the fibers vertically toward the surface and then produce perforating organs to develop the mycelium. The fungi are classified as keratinolytic when these invasion modes are detected. Furthermore, two individual phases of fungal keratinolysis have been reported from several studies (Fig.1.4). The first phase involves a frond-like mycelium and the second causes borers to form. All keratinophilic fungi are capable of growing on keratinous material and produce frond-like mycelia which are essential for fungal growth on complex material. Absorbing nutrients is the main function of the frond-like mycelium.

Some fungi can penetrate keratinous material by penetrating the substrate via the production of pressure (Błyskal, 2009). The ability of the keratinolytic fungi to breakdown keratin might be the result of several factors: extracellular keratinase, mechanical keratinolysis (mycelia pressure and/or penetration of the keratinous substrate) and sulphitolysis (reduction of disulphide bonds by sulphite excreted by mycelia) and proteolysis (Gupta and Ramnani, 2006).

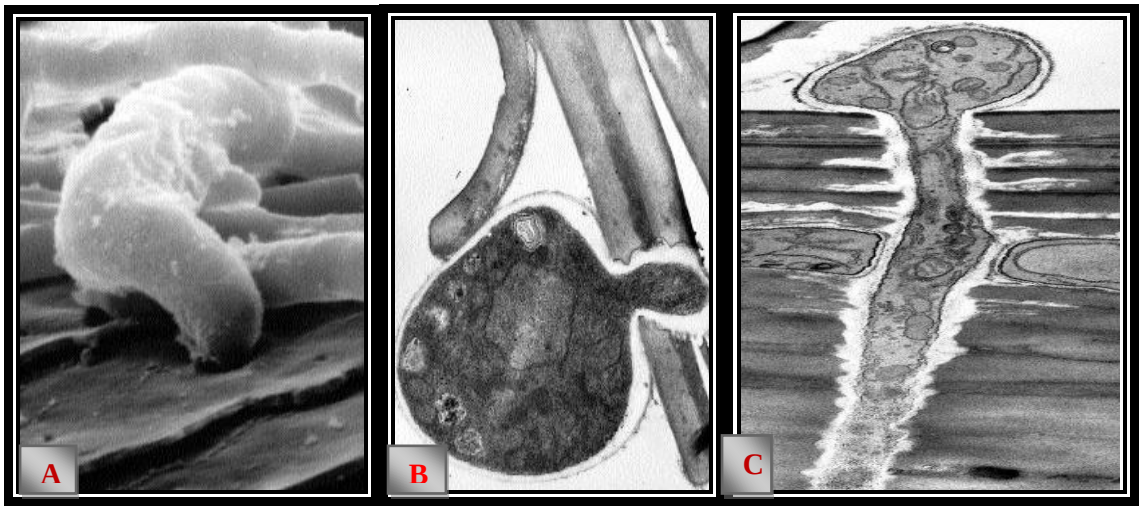


Figure 1.4: Development of boring hyphae (BH) using human hair.(A) The initial spot of BH on the surface of cuticular scales (CS), (B) young BH penetrates to the depth of two CS, (C) BH crosses the all cuticle section and the part of the cortex; source (Marchisio, 2000).

1.3.3.1. Keratinolytic potential of fungi

Several definitions of keratinolytic and keratinophilic fungi have been used for many years and the terms are often used as synonyms. Keratinolytic fungi are the fungi that breakdown keratin substrates completely (Kushwaha and Gupta, 2008). The term ‘‘keratinolytic’’ is now used to describe a group of microorganisms which produce specialized enzymes (keratinases) capable of attacking and decomposing keratin and which have pathogenic potential to humans. Keratinophilic species are able to easily use only digestible substances (products of partial decomposition of keratin, materials related to keratin). As a result, keratinolytic fungi live in communities together with keratinophilic fungi (Ulfig, 2005). Keratinolytic activity has been reported for several fungal species, such as *Aspergillus*, *Rhizomucor, Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum canis* (Tatineni *et al*., 2007).

1.3.3.2. Ecological importance of keratinolytic fungi

Keratinolytic fungi have the potential to degrade even the hardest substances such as keratin. Ecologically, keratinolytic fungi are very important where human and animal populations exert strong pressure on the environment (Filipello Marchisio *et al*., 1991). In highly populated and industrialized areas the importance increases, due to high organic and inorganic contamination. Keratinous substrates in soil are degraded by these fungi (biodegradation) (Ganaie *et al*., 2010). Keratinolytic fungi play the following important roles:

- The degradation of the keratinous substrates used in biotechnological processes, such as the bioremediation of waste in contaminated locations.
- Keratinolytic fungi can be used as bioindicators of environmental contamination.
- The cycling of the most highly stable animal protein on earth-Keratin (Ulfig, 2000).
- Keratinolytic fungi can also, importantly, act as pathogens of Man and animal (i.e. dermatophytes) (Gherbawy *et al*., 2006).

1.3.3.3. Classification of dermatophytes

Ecologically, dermatophytes are classified into three groups:

- Anthropophilic (literally people-loving, i.e. infecting humans).
- Geophilic (soil-loving).
- Zoophilic (animal-loving, i.e. infecting animals).

Geophilic and zoophilic dermatophytes usually produce self-limiting infections. However, anthropophilic species can cause minor inflammation which continues for a long time (Brouta *et al*., 2002). Dermatophytes fungi are classified in the genera *Epidermophyton, Keratinomyces, Microsporum,* and *Trichophyton* (Kaul and Sumbali, 1999). The majority of dermatophytes belong to the Arthrodermataceae and Onygenaceae families in the Ascomycetes. Almost all fungi grow on higher plants or their remains and survive saprophytically. In contrast, Arthrodermataceae and Onygenaceae are unusual in being associated with birds and mammals. Subsequently, these are considered true fungi which strongly degrade keratin and act as important pathogens for humans and animals (Kushwaha and Gupta, 2008).

1.3.3.4. Isolation of keratinophilic and keratinolytic fungi

Keratinophilic fungi are easy to isolate with hair baiting technique the (HBT). In addition, the isolation can be achieved using dilution plate methods (Sharma and Rajak, 2003). The brush culture technique has also been used to obtain clinical isolates (Abdel-Rahman, 2001). The first discovery of keratinophilic fungi from soil was with hair baiting technique which is the most common method used for the qualitative and quantitative isolation of these fungi from soil (Ali-Shtayeh and Jamous, 2000). The hair baiting method is better for the following reasons:

- Keratinolytic ability can be checked if the fungus grows on the natural keratin substrate.
- All keratinolytic fungi grow on most artificial media except some unusual strains which have special nutritional requirements.

- HBT yielded a slightly higher number of keratinophilic fungi (43 species) from soil than does the dilution plate technique (38 species).
- HBT is considered a more efficient method for the isolation of dermatophytes from soil. *Microsporum gypseum* and *Trichophyton ajelloi* have been isolated with HBT while no dermatophytes have been isolated with the dilution plate technique (Ali-Shtayeh and Jamous, 2000).

1.3.3.5. Detection of keratinolytic activity of fungi

If a fungus actively degrades keratin in the baits it is regarded as being keratinolytic. Some biochemical evidence is also needed for a fungus to be considered keratinolytic. Two approaches are used to determine if a fungus is keratinophylic:

i) **Morphological assessment**

A fungus is keratinolytic if it degrades keratin actively in the baits. This identification is not certain however, and further biochemical evidence is needed.

ii) **Biochemical assessment**

Several biochemical assessments have been reported in many studies such as release of keratinase and release products of keratin. Products of keratin e.g. cysteine, s-sulphocysteine and inorganic sulphate can be measured to detect keratinolytic activity in the culture filtrates. If these compounds are not detected in the culture filtrates, then the fungus does not have the ability to degrade keratin. Several keratinous substrates (commercial or native) can be used for this assay. Keratin azure (Sigma) for example, can be used to detect keratinolytic ability. Keratin azure is a blue compound which changes to colorless, indicating the keratinolysis (Sharma and Rajak, 2003).

1.3.4. The bacterial keratinolysis process

Microbial degradation of keratin can be achieved also by bacteria. The rate of this process is based on bacterial action and some factors such as moisture and temperature. Generally, proteins degrade into polypeptides, peptones, amino acids and some gases such as carbon dioxide, hydrogen sulfide, ammonia and methane. The process mainly includes sulfur containing amino acids such as cysteine, cystine. Desulfhydralation and decomposition can occur for these components via the action of bacteria and produce hydrogen sulfide gas, sulfides, ammonia, thiols and pyruvic acid. The most important proteolytic bacteria involved are genera of *Pseudomonas, Bacillus* and *Micrococcus* (Dent *et al*., 2004). Several bacteria have been isolated from soils and poultry waste and have proved to be good keratinase

producers and therefore the potential for use in biotechnological processes. Most keratinolytic bacteria are members of the *Streptomyces* and *Bacillus*. Among feather-degrading bacteria (Gram-positive bacteria) *Arthrobacter sp*., *Microbacterium sp* and *Kocuria rosea* have been identified as keratinolytic bacteria. Feather degradation can be achieved within 48 hours in most keratinolytic bacteria, notably *Microbacterium sp.* Some *Streptomyces* species are thermophilic and can degrade keratin at temperatures higher than 50 °C. Keratinase activity is recognized also in several species of *Bacillus* spp., including *B. licheniformis*, *B. subtilis, B. pumilus* and *B. cereus. Bacillus licheniformis* is able to decompose feathers completely as its keratinases often exhibit high activity. Keratin-degrading activity has been recognized in thermophilic and alkaliphilic isolates of *Bacillus* such as *B. halodurans*, and *B. pseudofirmus.* Recently, keratinase activity has been linked with Gram-negative bacteria such as *Xanthomonas maltophilia*, *Chryseobacterium sp.* and *Stenotrophomonas sp.* which have been isolated from chicken feathers as feather-degrading strains (Brandelli, 2008).

1.4. Microbial degradation of complex carbohydrates

Microorganisms can degrade simple substrates, such as sugars and amino acids, as well as more complex substrates such as pectin and chitin.

1.4.1. Degradation of pectin

The heterogeneous form of pectin requires several enzymatic activities. These enzymes, called pectin-degrading enzymes (polygalacturonases) are used in many industrial sectors involving wastewater treatment, food processing and textile industry.

1.4.1.1. Pectin substances

Pectin is considered as one of the most complex substrate (heteropolysaccharide) found in cell wall and middle lamella (helps to combine cells together) of fruits and vegetables (Favela-Torres *et al*., 2006). Pectins have been detected in fruits and vegetables such as apple, citrus and beet (Table 1.2).

Table 1.2: The percentage of pectin in some vegetables and fruit; adapted from (Jayani *et al*., 2005).

Fruit/vegetable	Tissue	Pectin substance (%)
Apple	Fresh	0.5–1.6
Banana	Fresh	0.7–1.2
Peaches	Fresh	0.1–0.9
Strawberries	Fresh	0.6–0.7
Cherries	Fresh	0.2–0.5
Peas	Fresh	0.9–1.4
Carrots	Dry matter	6.9–18.6
Orange pulp	Dry matter	12.4–28.0
Potatoes	Dry matter	1.8–3.3
Tomatoes	Dry matter	2.4–4.6
Sugar beet pulp	Dry matter	10.0–30.0

Fruit pectin consists of 1, 4- linked -galacturonan chain with branches of monosaccharides. The amount of pectin present and its molecular weight differs between fruits and vegetables (citrus > apple > beet)(Ovodov, 2009). The components of pectin include protopectin, pectin polysaccharides and associated galactans, arabinans and arabinogalactans. Protopectin is considered to be an insoluble complex molecule form which is found with cellulose and hemicelluloses in the cell walls of plants.

Pectin substances can be classified into four types (Ovodov, 2009):

(i) **Protopectin:** Insoluble pectin found in unbroken tissue. When protopectin degrades it releases pectin acids or pectin. Protopectin is the term used to describe insoluble pectin substrates.

(ii) **Pectin acid:** Soluble galacturonans composed of a small quantity of methoxyl groups.

(iii) **Pectinic acids:** Polygalacturonan chains composed of more than 0 and less than 75% methylated galacturonate units.

(iv) **Pectin:** Contains galacturonate units which are esterified with methanol (Jayani *et al*., 2005; Kashyap *et al*., 2001).

1.4.1.2. Importance of pectin substances

Pectin substances can be found in all higher plants. Pectin allows plant growth and cell wall extension. Pectins give plants resistance against drought and low temperatures and contribute in human nutrition as a food and fiber source (Ovodov, 2009). Pectin substances are added to several types of food because they are considered as natural stabilizing, gelling and thickening agents. In addition, they have nutritional benefits for the human diet due to the presence of nutritional fiber. Pectin has effects on human health, including reducing the level of cholesterol in the blood and deceleration of the absorption of glucose in obese and diabetic patients. Furthermore, citrus pectin is very active as anti-cancer agents in at all stages of cancer (Benoit *et al*., 2012).

1.4.1.3. Properties of pectin

Pectin is heteropolysaccharide found in all higher plants located in the middle lamella and cell wall. Basically, Pectin consists of two components; the main structure of pectin is homogalacturonan (HG). HG is linear polymer of α-(1–4) -linked D-galacturonic acid. It is formed by galacturonic acid (GalA) (α 1-4 linked). The most important feature of HG is the carboxyl group of galactoronic acid (Fig. 1.5).

The HG backbone can be interrupted by side chains of neutral sugar to create the rhamnogalacturon I (RGI) or rhamnogalacturon II (RGII) regions. RGI consists of 1, 4 linked D GalA and 1, 2 linked α L rhamnose with neutral sugars. The RGI region is considered as the site for neutral sugar (galactose, arabinose, glucose, and other sugars) attachment. In addition, the other chain side includes arabinogalactan (I and II) and xylogalacturons, mostly linked with protein. The linkages which result can be acid and alkaline stable (Wicker *et al*., 2014). In general, RGI region consist of repeating disaccharides of α-D-galacturonic acid and

(1,2)-α-L-rhamnose as backbone combine with neutral sugars mainly- galactose,D-xylose and L-arabinose.

1.4.1.4. Mechanisms of pectin degradation

Pectin substances can be degraded by two different mechanisms

- **Hydrolysis**

These mechanisms stimulate the hydrolytic cleavage of oxygen bridge with participation of water.

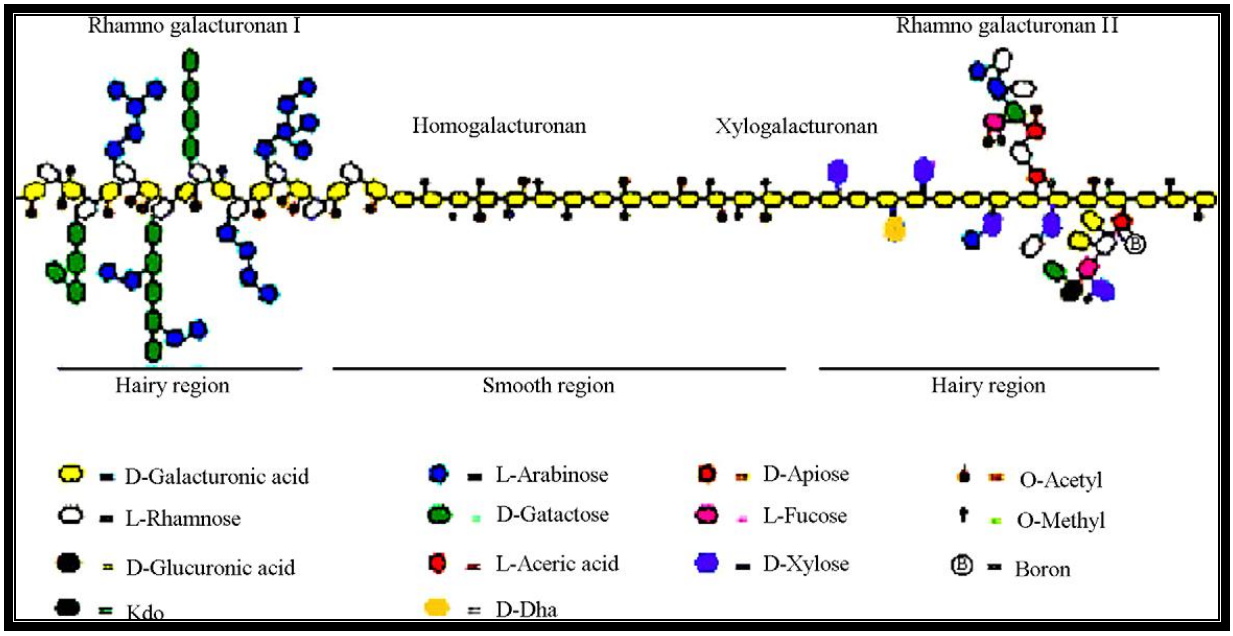


Figure 1.5: Structural units of pectin: source (Yadav *et al.*, 2009).

- **Trans-elimination lysis**

Basically, this mechanism cleaves the glycosidic bond without any contribution of water (Jayani *et al.*, 2005). The degradation of pectin can be achieved via pectinolytic enzymes (pectinases) (Fig. 1.6). The pectinolytic enzymes can be classified into two main groups according the mechanism of degradation:

1- **Pectinesterases** (**PE**): These enzymes enhance the de-esterification of pectin by removing methoxy residues such as pectin methylesterase (PME). PME is able to hydrolyze the methyl ester of galacturonide chain and release methanol.

$$\text{Pectin} + n\text{H}_2\text{O} \xrightarrow{\text{PE}} \text{pectate} + n\text{C}_2\text{H}_5\text{OH}$$

2- **Depolymerases:** Promote the cleavage of main chain in pectin. Depolymerases can be sub classified into two groups:

- **Polygalacturonases (PG):** These enzymes such as polygalacturonase and polymethylgalacturonase (PMG) cleave the glycosidic bonds by hydrolysis mechanism between two galacturonic acid residues.
- **Pectin lyases (PL):** Also called transeliminases and they achieve non-hydrolytic degradation of pectin polymer.

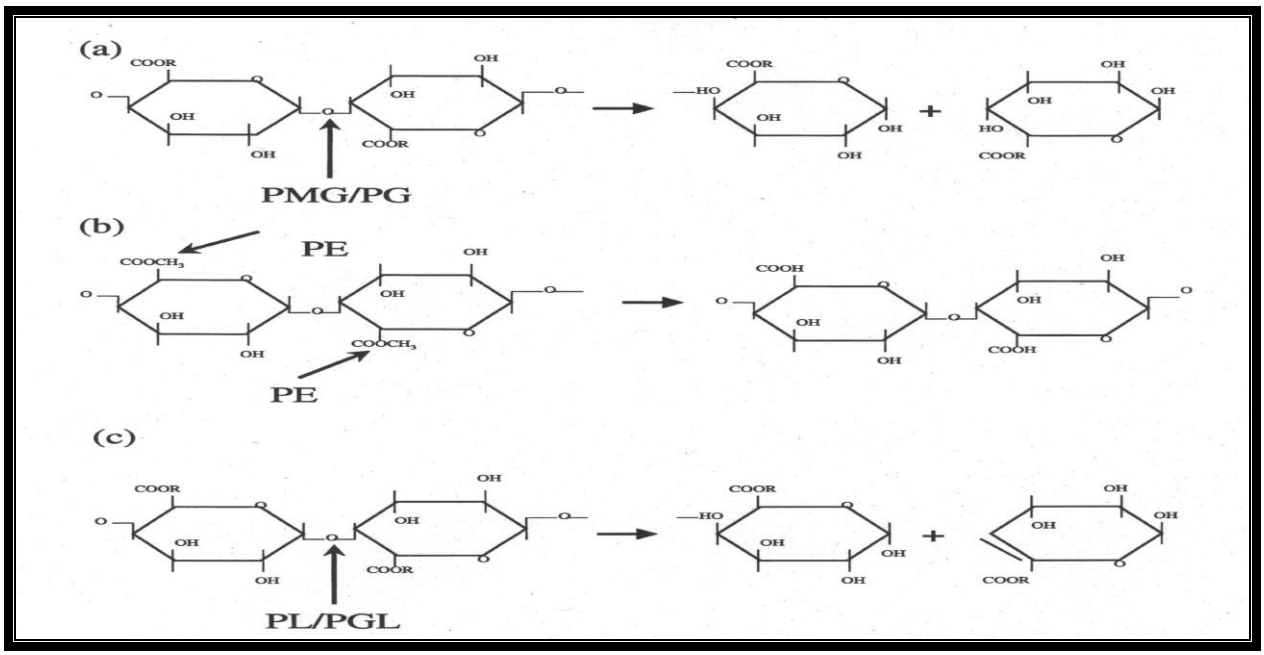


Figure 1.6: Pectinases types and the mode of degradation mechanisms (a) R=H for PG and CH_3 for PMG, (c) R=H for PGL and CH_3 for PL. The reaction point for pectinase shown by arrow. Source; (Gummadi and Panda, 2003).

These enzymes such as polymethylgalacturonate lyase (PMGL) and polygalacturonate lyase (PGL) cleave the glycosidic bonds by β-elimination between two methylated residues (Blanco *et al*., 1999).

polygalacturonate $\xrightarrow{\text{PGL}}$ unsaturated galacturonates.

polymethylgalacturonate $\xrightarrow{\text{PMGL}}$ unsaturated methyloligogalacturonates.

Pectinases can also be divided into Endo and Exo enzymes based on the type of action; if the

action is random the enzyme named is prefixed-Endo. In contrast, enzymes are prefixed Exo- if their calalytic action is terminal (Jayani *et al*., 2005).

1.4.1.5. Pectinases

Pectinases or pectinolytic enzymes, breakdown pectin derived from a wide range of plants. The classification of these enzymes is based on the preference of substrate such as pectin or pectin acid or oligo-D- -galacturonate. Also, the classification depends on the degradation mode (hydrolysis or transelimination) and the mode of cleavage (random [endo-pectinase] or terminal [exo-pectinase]). Pectinases are widely used in food applications such as clarification and extraction of vegetable oil and fruit juice. Most pectinases are considered polygalacturonases (PGases) and pectin lyases (PLases). Polygalacturonases (PGases) have endo and exo activities and they are hydrolytic depolymerases. The production of PGases has been shown by more than 30 different genera of bacteria, yeasts and moulds. These microorganisms include genera of *Erwinia, Bacillus, Saccharomyces, Kluyveromyces, Aspergillus, Penicillium* and *Fusarium*. Most of the strains used for enzyme production studies are isolates of *Aspergillus*, *Penicillium* and *Erwinia* (Favela-Torres *et al.*, 2006). Microorganisms generally release only one type of each pectinase. However, there are some which produce different types of pectinases in order to breakdown pectin (Latif and Sohail, 2012).

1.4.1.6. Microbial production of pectinases

Pectinases are produced by variety of microorganisms that promote the breakdown of glycosidic bonds of pectin substrate. These microorganisms include bacteria, yeasts and fungi. Among the genera of pectinase-producing fungi are species of *Aspergillus* which breakdown monosaccharides or oligosaccharides released from polymers during the initiation of fungal growth. Pectinases differ according to the species of fungi. For example *Rhizopus spp.* produce enzymes which breakdown only the homogalacturonan fraction of pectin, whereas *Aspergillus* hydrolyse all elements in pectin. *Aspergillus* species are extensively used to produce large amounts of these enzymes (Martínez-Trujillo *et al.*, 2009). Furthermore, *Aspergillus niger* and *Aspergillus oryza* are used for enzyme production as industrial fungi. Commercially, filamentous fungi produce the majority of pectinolytic enzymes. Filamentous fungi are capable of breaking down the polysaccharide component of the plant cell wall efficiently and convert them to monosaccharides which they then use as nutrients. Pectinolytic filamentous fungi include (i) ascomycetes such as *Aspergillus nidulans*

and *Trichoderma virens* (ii) basidiomycetes such as *Phanerochaete* and *Chrysosporium* (Benoit *et al*., 2012).

Bacterial pectinases are considered to be the most important pectinase and are used in many industrial sectors including in food, paper and pharmaceutical production. *Bacillus licheniformis* and *Staphylococcus aureu*s have been identified as pectinase producer by Venkata Naga Raju and Divakar (2013).

1.4.1.7. Applications of pectinase

The commercial application of pectinases began in 1930 to prepare fruit juices and wines, but scientists started to use a huge range of enzymes only in 1960s when the chemical makeup of nature of plant tissues was fully determined. In 1995 the estimated cost of all industrial enzymes was $1 billion, pectinase was assessed to the value of $75 million (Kashyap *et al*., 2001). Pectinases are important in several applications such as:

- **Extraction and clarification of fruit juice**

This process is the major industrial application of pectinase. Based on this process, a mixture of pectinase and amylase is used to clarify fruit juices; lemon oil (citrus oil) can also be extracted using pectinase.

- **Textile processing**

A mixture of pectinases, amylases, lipases, cellulases and hemicellulases has been used in the bioscouring process to remove non-cellulosic parts from the fibres. In this purpose, pectinases have been used without side effects on cellulose degradation.

- **Waste water treatment**

The food industry, such as vegetable food processing, discards pectin into the marine environment as waste water. Pectinases are able to remove pectinaceous materials from these environments.

- **Animal feed**

Pectinases are used in animal feed production because they reduce viscosity. As a result, the absorption of nutrients increase via the breakdown of non-biodegradable fibers (Jayani *et al*., 2005).

1.4.2. Degradation of alginate

The oceans of the world involve several kinds of habitats that include a variety of life-forms (Zhang and Kim, 2010) including seaweeds (Fleurence, 1999).

1.4.2.1. Seaweed (macroalgae)

Macroalgae are marine plants that can grow to huge size (up to 60 m in length). Seaweeds have principally been recycled to produce a number of complex organic molecules such as human foods, cosmetics, and fertilizers, and for the extraction of industrial gums (phytocolloids) and chemicals. Seaweed can also act as a source of a variety of different materials for the production of phytocolloids such as agar, carrageenan (derived from Rhodophyta) and alginates (derived from Phaeophyta) (Ross *et al*., 2008). Figure 1.7 shows various forms of seaweed which can be converted to many different products including: medicines, and agricultura1 products, paper and for the production of biogases (Speed, 2005).

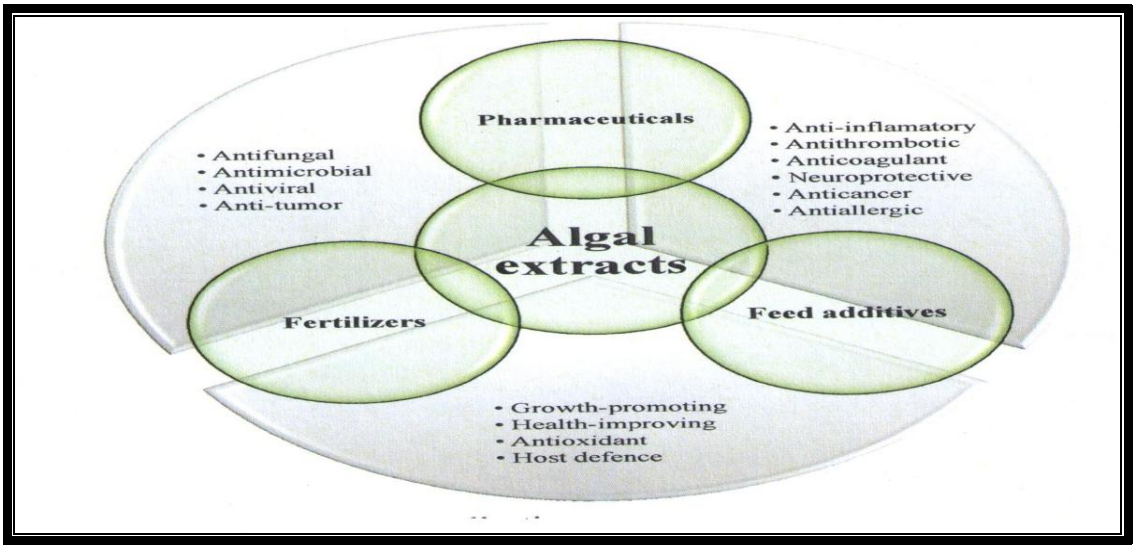


Figure 1.7: Activities of algal extracts and their application (Chojnacka *et al*., 2012).

Algae are classified into the cyanobacteria (Cyanophyta), green algae (Chlorophyta), brown algae (Phaeophyta) and the red algae (Rhodophyta). Phaeophyta have been acknowledged to include two orders, the Laminariales (kelps) and the Fucales (fucoids) (Steinberg, 1989). Kelp species occur in several countries, including the UK. The main species include *Laminaria digitata*, *Laminaria hyperborea*, *Laminaria ochroleuca*, and *Laminaria saccharina* (Ross *et al*., 2008). A wide variety of commercial sources of industrial alginic acid are produced from *Laminaria* (Wang *et al*., 2008). Seaweeds play a significant role in biotechnology; active compounds are converted from the biomass of algae to the liquid stage include polysaccharides, proteins, polyunsaturated fatty acids, pigments, polyphenols, minerals, plant growth hormones etc.

1.4.2.2. Diversity of marine microbial enzymes

Marine microorganisms have received increasing attention because they are capable of producing novel enzymes. These enzymes are generally more stable and active than the enzymes derived from plants or animals. A number of advantages result from the use of these enzymes in the production of pharmaceuticals, food additives and chemicals. To date, a variety of enzymes have been isolated, with attractive properties, from marine bacteria, actinomycetes, fungi and other marine microorganisms (Zhang and Kim, 2010), and a wide variety of seaweeds contain many different polysaccharides (sulphated polysaccharides, Laminarin and Alginates).

1.4.2.3. Alginate

Currently, alginates are used in food modification as a food additive because they have high viscosity and gelling features. Alginates can be found in brown macroalgae and some bacteria. Marine macroalgae such as *Laminaria digitata , Laminaria hyperborea, Laminaria japonica, Macrocystis pyrifera, Ascophyllum nodosum, Eclonia maxima, Lessonia nigrescens, Durvillea Antarctica* and *Sargassum* sp. are considered the most important sources of alginates for use in commerce (Kim *et al*., 2011). Some species of bacteria are able to synthesize alginates, such as *Azotobacter chroococcum, Azotobacter vinelandii* and *Pseudomonas aeruginasa* (Gacesa, 1992). Alginate is a complex molecule, which involves linear polymer of α-L-guluronic acid (G) and β-D-mannuronic acid (M) (Fig. 1.8). Its structure depends on monomer ratios and the distribution of monomers into homopolymeric blocks (GG or MM) or heteropolymeric blocks (MG) (Fig. 1.9). As a result, the alginate structure is highly changeable. Figure 1.8 shows the monomers of alginate linked by 4-0-glycosidic bonds. The cross-link can be chemically degraded by acid hydrolysis or alkali-catalyzed β-elimination. Alginate degradation is the process achieved by enzymes which disrupt other polysaccharide linkages in the wall and are not specific for alginate linkages (Doubet and Quatrano, 1982).

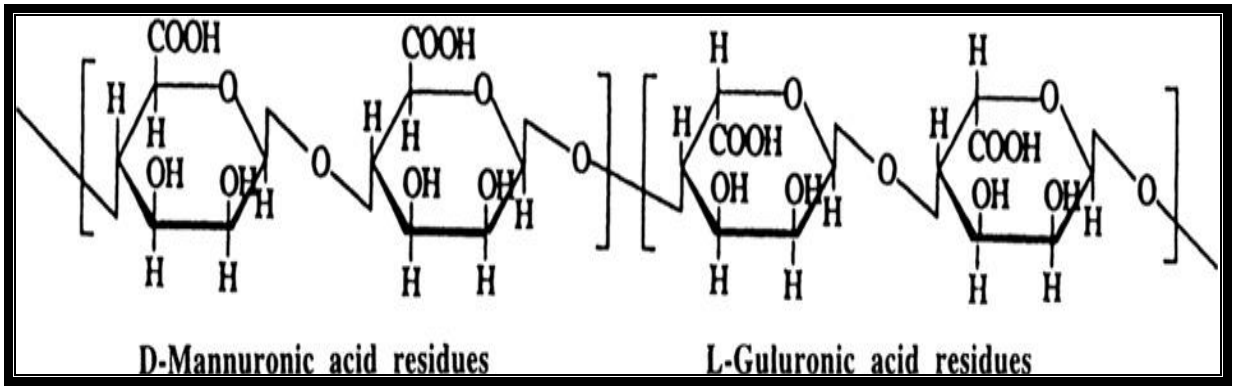


Figure 1.8: Structural units of alginate. Source: (Gombotz and Wee, 1998).

Basically, the monomers of uronic acid are combined together to generate:

- Polymannuronate (polyM-block).
- Polyguluronate (polyG-block).
- Random co-polymer (polyMG-block) (Fig. 1.9) (Kim *et al*., 2011).

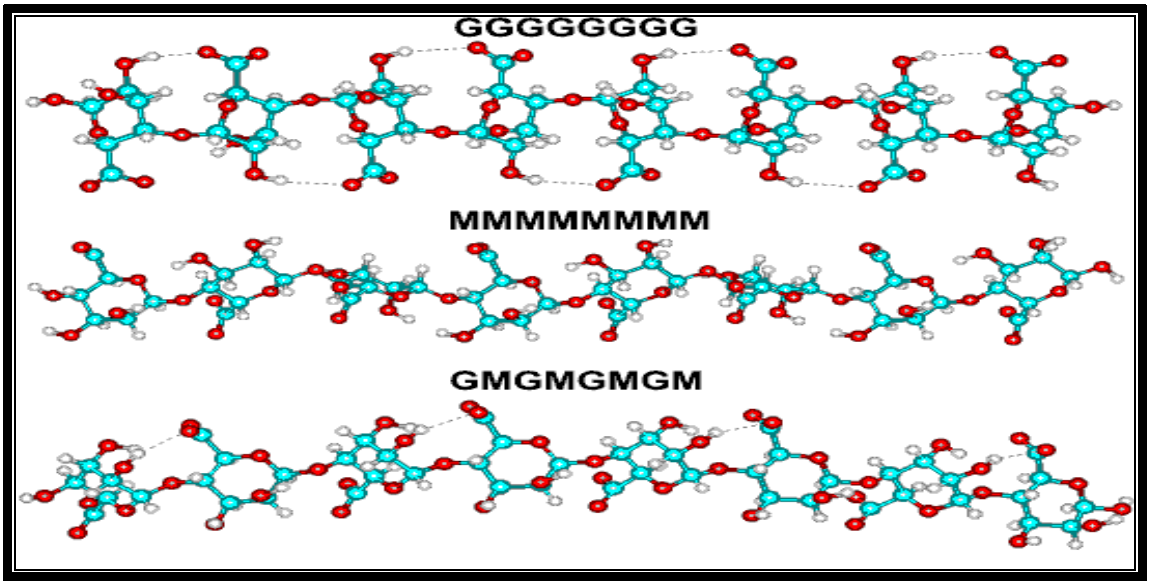


Figure 1.9: Molecular Structure of alginate http://www1.lsbu.ac.uk/water/

The salts of alginic acid can be considered alginates; these include ammonium alginate, sodium alginate, potassium alginate and calcium alginate. The molecular structure of alginic acid is variable and depends on the ratio and distribution of the monomers in the homopolymeric (GG or MM), or in heteropolymeric units (MG) (Abdel-Hafez *et al*., 2008).

1.4.2.4. Alginate Lyase

Microorganisms release a range of extracellular enzymes (lysozymes, proteases and nucleases) which can begin the degradation of complex compounds such as polysaccharides.

Alginate lyases are extracellular enzymes secreted by microbes, plants and animals. Alginate lyases are classified according to their preferred substrate specificities:

- Poly G-specific lyases preferentially degrade poly G-block.
- Poly M-specific lyases have a preference for poly M-block (Kim *et al*., 2011).

The biosynthesis and biodegradation of alginates is considered the main function of alginate lyase. Numerous alginate lyases have been discovered from brown algae, marine and soil microorganisms. Alginate lyases occur in non-alginate-synthesizing and alginate-synthesizing organisms. In the non-alginate-synthesizing organisms; alginate lyases play important roles in assimilation of alginate as a carbon source. More than one alginate lyase (responsible for the degradation of alginate) has been recognized in marine and soil microorganisms (Kim *et al.*, 2011). Alginate lyases mostly are endolytic enzymes which decompose internal glycoside linkages, while a few alginate lyases are exolytic; this type of alginate lyase has been isolated from *Sphingomonas sp.*(Suzuki *et al*., 2006).

1.4.2.5. The pathway of alginate degradation

This process is highly dependent upon the activity of microorganisms, and there are two pathways which convert alginate into useful products. Alginase enzymes hydrolyse 1-4 glycosidic linkage using a β-elimination reaction:

- **Endo-type alginate lyases**

These can exhibit polyM-, polyG-, or polyMG-specific activity. Alginate polymer is decomposed to produce oligomer with unsaturated uronic acid at the non-reducing ends (4-deoxy-L-erythro-hex-4-enepyranosyluronate).

- **Exo-type alginate lyase**

The alginate oligomers then undergo further depolymerisation into unsaturated monosaccharide by the action of exo-type alginate lyase. The monosaccharide is then non-enzymatically converted to 4-deoxy-L-erythro-hexoseulose uronic acid (DEH), and then the α-keto acid is converted to 2-keto-3-deoxy-Dgluconic acid (KDG). The KDG is then converted to 2-keto- 3-deoxy-6-phosphogluconic acid (KDPG). Finally, the KDPG is split into pyruvate and glyceraldehydes-3-phosphate (Kim *et al*., 2011).

1.4.2.6. Microbial degradation of alginate

Alginate lyases have been isolated from several species of bacteria including:

- Marine bacteria such as *Halomonas marina, Photobacterium* sp. *Vibrio* sp. and *Beneckea pelagia.*

- Gram-negative soil bacteria such as *Pseudomonas aeruginasa, Azotobacter vinelandii, Azotobacter chroococcum, Alteromonas sp.* and *Klebsilla pneumonia.*
- Gram-positive soil bacteria such as *Bacillus sp., B. circulans* and *Clostridium alginolyticu* (Wong *et al*., 2000).

Alginate lyases from bacteria are mostly extracellular (Gacesa, 1992). Marine fungi, such as members of the Phycomycetes, Ascomycetes, yeasts and Hyphomycetes have also been investigated for this ability (Schaumann and Weide, 1995).

Alginate lyases have been detected in only a few fungal species including, *Dendryphiella salina, Dendryphiella arenaria* (Deuteromycetes), *Corollospora intermedia* (Ascomycetes) and *Asteromyces cruciatus* (Deuteromycetes) (Shimokawa *et al.,* 1997). Alginate degradation by *D. salina,* has been investigated and confirmed by Wainwright (1980) while the efficiency of alginate degradation by *D. salina* and *D. arenaria* was further confirmed by Wainwright and Sherbrock-Cox (1981) . Alginate breakdown by *Asteromyces cruciatus* and *D. salina* occurs in two stages:

i) **Depolymerisation**

This phase is enhanced by an endo-hydrolase and the most important feature is a rapid decrease in viscosity and increase in reducing substances.

ii) **Formation of 4-deoxy-L-erythro-hex-4-ene pyranosyluronate**

This stage is promoted by alginate lyase and it includes a minor further decrease in viscosity and the rapid formation of 4-deoxy-L-erythro-hex-4-ene pyranosyluronate and matching oligosaccharides (Gacesa, 1992).

Alginate lyase can be used to enhance the effectiveness of antibiotics (e.g.gentamicin) in the respiratory tract.

1.4.3. Degradation of chitin

Chitin is an insoluble homopolymer which consists of N-acetylglucosamine (NAG) linked together via β-1,4-glycosidic bonds (Kishore and Pande, 2007) (Fig. 1.10). Chitinous waste is produced largely by shrimp processing industries. The current use of chemical treatment produces hazards such as release of toxic chemicals including HCl, acetic acid and NaOH into water courses. There is however, the need for alternative approaches to the treatment of this waste, including enzyme degradation. Chitin is found in the exoskeletons of crustaceans e.g. crabs, shrimp, lobsters, insects, worms, fungi and mushrooms. It is a bioabsorbable material and can be used in antibacterial bandages. Chitin also has several

application in many sectors such as agriculture, food technology, material science, microbiology, wastewater treatment, drug delivery systems and tissue engineering (Kandra *et al*., 2012).

Chitin is insoluble in water due to intermolecular hydrogen bonds between its monomers of chitin. The percentage of nitrogen found in chitin is 6–7% while in its deacetylated form (chitosan) is 7–9.5%. Three forms of chitin can be recognized: α, β, and γ chitin. The α-form can be obtained from crab and shrimp shells and it is commonly distributed (Kandra *et al.*, 2012). Chitosan is type of chitin which is totally or partially deacetylated. Deacetylation of chitin is artificial (a chemical processes); deacetylation produces chitosan (Wang *et al*., 2009). Chitosan is the major part of soil fungal walls (Zygomycetes) and is produced by several microbes include bacteria e.g. *Myxobacter, Sporocytophage, Arthrobacter, Bacillus* and *Streptomyces*. It can also be produced by fungi such as *Rhizopus, Aspergillus, Penicillium, Chaetomium* and various Basidiomycetes (Gooday, 1990).

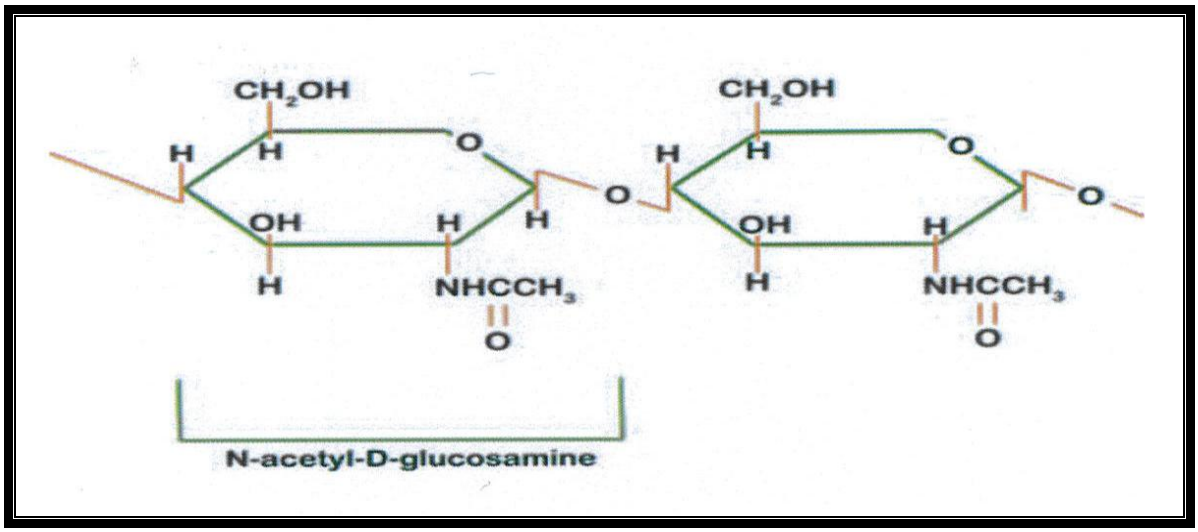


Figure 1.10: Molecular structure of chitin. Source; (Severgnini, 2006).

1.4.3.1. The Chitin degradation pathway

Chitinases are selective enzymes which degrade chitin using hydrolysis of the β-1, 4-glycosidic bonds that link monomers of chitin (Fig. 1.11). Chitinase have antifungal activity against wide range of microorganisms. Microorganisms produce two types of chitinase exo- and endo-enzyme. The classification of exo- and endo-enzyme based on nature of substrate. For example, *Streptomyces* chitinase is able to breakdown the pure β-chitin only (from the

non-reducing ends) to produce diacetylchitobiose, while colloidal chitin is decomposed to a mixture contains oligomers and diacetylchitobiose (Shaikh and Deshpande, 1993). The microbial mechanism involved includes the hydrolysis of glycosidic bonds and this action is known as chitinolytic which involves two steps achieved by two chitinase enzymes:

- Exo-chitinase which breaks down the units of polysaccharides chain (diacetylchitobiose) at the non-reducing ends.
- Endo-chitinase which breakdown the glycosidic bonds randomly.

The main products are diacetylchitibiose (called chitobiose) with triacetylchitibiose. These activities are based on the nature of substances for example, a mixture of oligomers and diacetylchitibiose can be produced from degradation of colloidal chitin (Gooday, 1990).

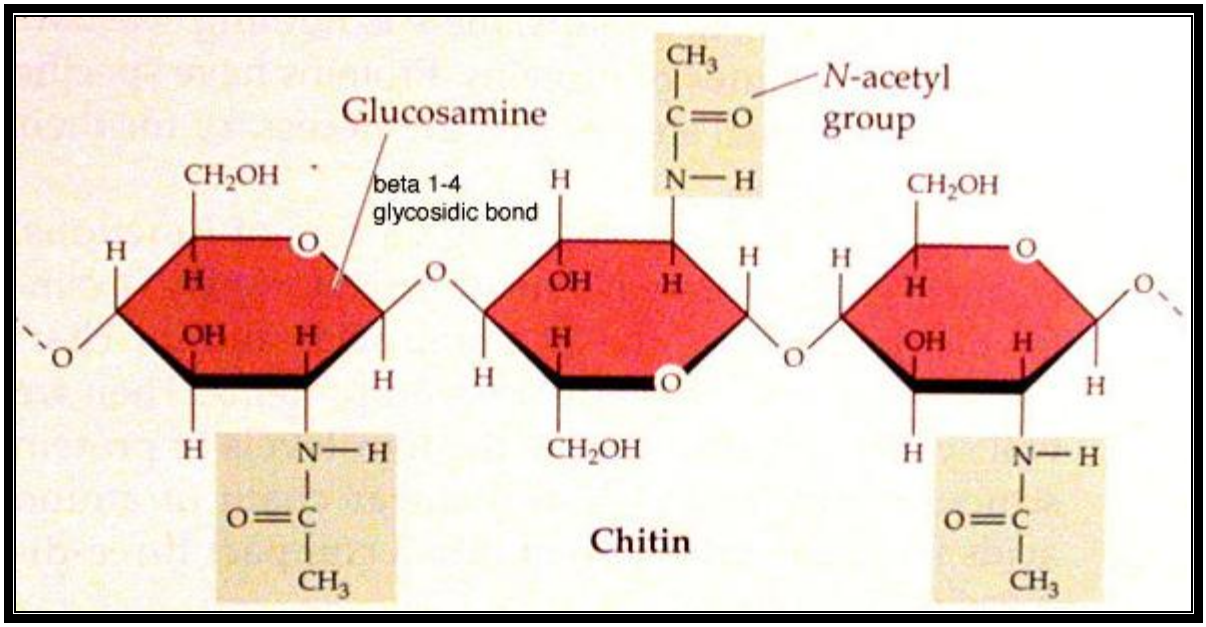


Figure 1.11: Diagram of β-(1→4) linkages of chitin. Source; (Pasayat, 2007).

1.4.3.2. Microbial degradation of chitin

Marine chitinolytic genera include species of *Photobacterium*, *Aeromonas*, *Bacillus* and *Chromobacterium.* Chitinolytic bacteria have also have been detected in fresh water such as *Serratia, Chromobacterium, Pseudomonas, Flavobacterium* and *Bacillus.* While in the soil, chitinolytic bacteria include species of *Pseudomonas, Aeromomas, Cytophage johnsonae, Arthrobacter* and *Bacillus.* Fungi are major chitinolytic microorganisms and are capable of degrading chitin efficiently in the soil. They are mostly Mucorales, such as *Mortierella spp*. and Deuteromycetes and Ascomycetes, e.g. species of *Aspergillus, Trichoderma, Verticillum*

and *Penicillium.* Chitinolytic fungi called chytrids including *Chytriomyces spp.* and *Karlingia astereocysta* are also found in freshwaters (Gooday, 1990).

1.4.3.3. Use of chitinases in biotechnology

Chitinases can be used as:

- **Biocontrol agents**

For example *Aeromonbas caviae* can be used to control *Rhizoctonia solani* infection in cotton plants and also to control *Seclerotium rolfsii* in bean fields. Inoculation with chitinolytic microorganisms with the addition of chitinous waste to the soil also produces effective biocontrol of *Verticillum dahlia* and *Fusarium oxysporium* in beans and cotton fields. Finally, *Trichoderma harzianum* produce a chitinase which inhibits the elongation of plant pathogen hyphae, including *Fusarium solani*, *Botrytis cinerae* and *Cotricum rolfsi* (Felse and Panda, 2000).

- **Degradation of fish waste**

During the bioconversion of shell fish waste, *N*-acetyl-D-glucosamine with other important products can be produced. *N*-acetyl-D-glucosamine is considered a very beneficial monomer and can be used in many industrial sectors particularly in food production, e.g. of sweeteners and growth factors (Felse and Panda, 2000).

1.5. Identification of bacterial and fungal decomposers using molecular approaches

Molecular approaches such as polymerase chain reaction (PCR) are helpful tools for assessing the structure and function of microbial communities. PCR has become a familiar tool for the discovery and classification (taxonomy) of microbial species. Extraction of DNA is significant part of this approach, consequently DNA should be provided in enough quantity and purity (Osama *et al*., 2011). Molecular analysis of several microbial genomes with the use of PCR based technique is extremely useful. For example, the sexual and asexual stages of keratinolytic fungi are considered major difficulties in fungi identification, since morphologically, both stages can be quite similar. As a result, the identification of genera or species becomes very difficult without knowledge of both stages. DNA based identification techniques are useful for those fungi which are difficult to decide morphologically. Also, the use of molecular tools for species identification is beneficial because many fungi do not produce spores which are considered fundamental in fungal species identification (Sharma and Rajak, 2003). Basically, the major advantage of PCR that it can multiply a small amounts of DNA to billion times in a few minutes to a few hours. The reaction of PCR based

on three steps denaturation, primer annealing and primer extension (Hadidi and Candresse, 2003). Gene fragments or populations of rRNA genes can be generated by PCR amplification. Cell lysis and DNA extraction are the initial stage in the analysis of rRNA genes. The microbial population can be represented by population of gene fragments. These genes encode the small subunit (SSU) of ribosomal RNA which have been analysed in various molecular approaches. For the analysis of bacterial populations, the 16S rRNA gene is commonly used. However, 18S rRNA genes and internal transcribed spacer (ITS) regions is widely utilized for fungal population analysis and it is an efficient method in molecular fungal identification (Prosser, 2002).

1.6. The 18S rRNA gene

The 18S rRNA gene markers can be used in various applications such as molecular phylogenetic analyses (Meyer *et al*., 2010). Most eukaryotes including fungi have 80S ribosomes involve two subunits called large (60S) and small (40S) subunits. Each subunit consists of rRNA and a number of associated proteins. The large subunit including 28S, 5.8S and 5S rRNA molecules while the small subunit including the18S rRNA molecule. The rRNA molecules are also split by the ITS (internally transcribed spacer) regions, which are very changeable in length and sequence (Kennedy and Clipson, 2003). Fungal population has been analysed using molecular techniques. There are some studies that have used the small subunit rRNA (18S rRNA) for identification of fungal populations. The limitation is that this region has less useful information than 16S rRNA genes, as used to identify prokaryotes. Some researchers have analysed the regions of ITS but the problem is the shortage of fungal sequence information in the databases (Prosser, 2002). In addition, 18S rRNA gene sequences are only capable to determine taxonomic classes to the level of genus. Fungal 18S rDNA and ITS sequences taxonomic resolution is exclusive due to the present accessibility of information in databases (Anderson *et al*., 2003). Currently, this problem has decreased and the molecular identification has become more rapid (Prosser, 2002).

1.7. Aims of the study

The first aim of this research was to isolate keratinophilic fungi from soil (using hair and wool as baits) and from waste contaminated feathers, followed by the determination of the fungal degradation of keratin. Molecular techniques includes fungal gene sequencing were used to identify and characterise keratinolytic fungi. In addition, SEM studies were conducted to enable visualization of fungal keratin degredation on hair and wool (Chapter 2).

The second aim of the study was to determine the microbial degradation of apple pectin after screening of pectinolytic fungi and bacteria. Again identification was made using molecular methods. A further aim was to evaluate pectin as an antibacterial material against bacteria causing wound infections (Chapter 3).

Marine fungi and bacteria were isolated from detritus and decaying fronds of two brown algae namely, *Laminaria* and *Fucus*. Enzymic alginate degradation was studied in order to determine the bacterial and fungal degradation of this substrate. Identification of the marine fungi and bacteria using the molecular methods was again conducted (Chapter 4).

A further aim was to isolate chitinolytic bacteria and fungi which can breakdown chitin in the form of colloidal chitin. In addition, to detect chitinase activities of these isolates in broth medium (Chapter 5).

Finally, four enzymes (keratinase, alginate lyase, pectinase and chitinase) were isolated from certain bacteria using ammonium sulphate in order to determine the molecular weight of these proteins via SDS polyacrylamide gel electrophoresis (SDS-PAGE) technique. Also, liquid chromatography mass spectrometry (LC-MS/MS) analysis was used to identify three enzymes namely; keratinase, pectinase and chitinase; their properties were then compared to Swissport/Uniprot and NCBInr databases (Chapter 6).

CHAPTER 2

CHAPTER 2
STUDIES ON MICROBIAL DEGRADATION OF KERATIN

2.1. Introduction

Keratinous materials (from different industries) containing insoluble keratin, i.e., hair, nails, horns, hoofs, scales and wool all reach the environment, and are solid materials which resist degradation due to their disulfide bonds and cross-linkages. Keratins are classified into hard and soft keratins according to the sulphur content. Hard keratin can be found in feathers; hair, hoofs and nails which contain large amounts of disulfide bonds. Soft keratins are found in callus and the skin and are "soft" due to low amounts of disulfide bonds; they are also more flexible.

Keratinous waste can be decomposed efficiently by bacteria, actinomycetes and fungi due to the release of keratinases. The degradation of keratinous wastes seems to be a common process in Gram-positive bacteria such as *Microbacterium, Bacillus, Kocurica, Lysobacter* and *Nesternokia*. Keratin degradation has also been reported in a small number of Gram-negative bacteria such as *Chryseobacterium, Xanthomonas* and *Stenotrophomonas*. Keratinase production in bacteria has been studied in a few isolates such as *Bacillus licheniformis* and *B. subtilis.* These species have reached commercial use due to their high efficiency at degrading feather keratin. Keratinolytic fungi include genera of *Alternaria ,Chrysosporium, Aspergillus, Cladosporium, Fusarium, Geomyces, Penicillium, Urocladium, Scopulariopsis, Sepedonium,* and *Doratomyces.* Keratinase from fungi, bacteria and actinomycetes have optimum pH range between neutral to alkaline, although a few fungal keratinases have activity at pH more than 12, being more common at pH 6 to 9 (Gupta and Ramnani, 2006). Keratinolytic fungi exhibit a marked commercial potential in keratin degradation.

2. 1.1. Evidence for keratinolysis in nature

Although keratins are resistant to degradation keratinous waste, such as feathers do not accumulate in nature. Keratin hydrolysis can be performed by two mechanisms: the first step being sulphitolysis which leads to the reduction of disulphide bonds. Keratin is attacked by sulphitolysis of disulphide bonds, the products being peptides of sulphocystein, sulphate, thiosulphate and cystein:

$$\text{Cys-S-S-Cys}+HSO_3^- \Rightarrow \text{Cys-SH}+\text{Cys-SSO}^-_3$$

The second step in keratin degradation is proteolysis which includes the activity of keratinase (Onifade *et al*., 1998).

2.1.2. Industrial and medical applications of keratinase

Microbial keratinases are important in biotechnological processes such as:

- Pharmaceutical industries, feed, fertilizer and detergent production. However, the main application of keratinases is in the production of feather meal as a cost-effective and nutritious product (Gupta and Ramnani, 2006).
- The leather industry use keratinase, rather than chemicals in dehairing hides (Kushwaha and Gupta, 2008).
- Keratinase is added to feather meal in animal feed; this improves digestibility and avoids cooking feathers at high temperature.
- To clean drains blocked from keratin waste.
- Recently, keratinase has been used to produce vaccines against dermatophytosis.
- Fungal keratinases from poultry-waste degradation have the potential to generate natural gas for fuel (Kushwaha and Gupta, 2008).

The aims of this work reported in this Chapter are:

i) To isolate keratinophylic fungi from soil (using hair and wool as baits) and contaminated feathers.

ii) To determine the fungal and bacterial degradation of keratin, an important keratinolytic process in soil.

iii) To identify keratinolytic fungi using molecular identification techniques.

2.2. Materials and methods

An agricultural soil was collected from different sites in Sheffield region, mixed well together, placed in clean plastic bags and then transferred to the laboratory. Suspension consisting of the soil sample and ultrapure water was made in order to determine pH (10 grams soil in 10 ml ddH_2O and shaking them for 30 minutes on a shaker (150 rpm); the pH was determined using a pH meter.

2.2.1. Isolation of keratinophylic and keratinolytic fungi by hair-baiting technique

Keratinolytic and keratinophytic fungi were isolated from agricultural soil using the hair-baiting technique.

2.2.1.1. Keratinous substrates

Two native keratinous substrates were used in the hair-baiting technique, collected randomly from:

(i) Freshly collected human hair.

(ii) Sheep wool obtained from fields in the Sheffield region.

The substrates were placed in clean plastic bags and transferred to the laboratory.

2.2.1.2. Hair-baiting technique (HBT)

The hair-baiting technique was used to isolate keratinophylic fungi from soil using hair and wool as baits. Natural wool and hair were degreased using warm water and soap, cut into small fragments (2-3 cm) and sterilized using ether. Approximately 50g agricultural soil was placed into empty sterile containers and covered with sterile film containing holes. The soil with hair or wool was moistened with 10-15 ml of sterile distilled water then incubated at 25 °C for four weeks. The soil in containers (two for hair and two for wool) was moistened with sterile distilled water. The containers were regularly checked for the appearance of mycelium on the baits.

2.2.1.3. Dilution plate methods

After four weeks incubation, baited Soil (10g) was suspended in 100 ml of sterile distilled water, shaked for 15 min on an orbital shaker and then diluted serially (Madigan *et al*., 2003). A sample (0.1 ml) was spread on the surface of Sabouraud dextrose agar medium (SDA) (Sigma). SDA consisted of 10g peptone, 40 g dextrose and 15g agar in 1L of dH_2O at pH 5.2. SDA was sterilized by autoclaving at 121°C for 15 minutes. After sterilization, penicillin-streptomycin (0.5mg/ml) (Sigma) was added to prevent bacterial growth. The plates were incubated at 25°C for 7 days using 3 plates for each sample. The colonies developed on SDA were purified to use for keratinolytic assays.

2.2.1.4. Scanning electron microscopy (SEM) studies on morphology of keratin degradation

This technique aimed to view different stages of fungal colonization on baited wool and hair as keratinous substrates. Mycelia produced from the hair-baiting technique (HBT) as described above were harvested in sterile Eppendorf tubes and fixed in 3% glutaraldehyde in 0.1 M phosphate buffers for 4 hours at 4°C, then washed two times in 0.1M phosphate buffer at 15 intervals at 4°C. Fixation of samples was performed using 2% osmium tetroxide

aqueous for 1 hour at 25°C followed by repeating this step again. The samples were then dehydrated in serial dilutions of ethanol 75%, 95% and 100% three times. Finally, absolute ethanol (final step of 100% ethanol) was dried over anhydrous copper sulphate for 15 min. The samples were digested in absolute hexamethyldisilazane for 30 min in order to dry the samples using hexamethyldisilazane (HMDS). The samples were then left overnight to air dry. The dry samples were mounted on 12.5mm diameter aluminium stubs, and linked with Carbon-Sticky Tabs and coated with approximately 25nm of gold in an Edwards S150B sputter. Finally, the samples were viewed under a Philips XL-20 Scanning Electron Microscope at an accelerating voltage of 20Kv (Alnaimat, 2011).

2.2.2. Isolation of keratinophylic and keratinolytic fungi from feathers

Feathers were obtained from fields in the Sheffield region, placed in clean plastic bags and transferred to the laboratory. This method was aimed to isolate keratinophylic and keratinolytic fungi from samples without using soil (Gherbawy *et al*., 2006). Several samples of feather were inoculated directly into Sabouraud Dextrose Agar (SDA) medium containing penicillin-streptomycin (0.5mg/ml) at pH 5.2. The plates were incubated at 25°C for 1 week using 3 plates for each sample. The colonies developed on SDA were then purified for use in keratinolytic assays. All fungal isolates were stored on SDA slants at 4°C and the frequency of isolates was determined by use:

$$\%\ frequence = \frac{\text{Number of isolates of a fungus}}{\text{Total number of isolates}} \times 100$$

2.2.3. Fungal keratinolytic assay in solid medium

All isolates from wool, hair and feather were used in keratinolytic assay. However, extra laboratory isolates were also tested:

- *Trichophyton mentagrophytes* (a dermatophytic fungus) was obtained from G10 laboratory in Molecular Biology and Biotechnology Department (isolated from soil).
- *Unknown fungus* was obtained from G10 laboratory in Molecular Biology and Biotechnology Department (isolated from desert).

2.2.3.1. Keratinous substrate

All strains were tested using a commercial keratinous substrate is called Keratin Azure (Sigma) as carbon and nitrogen source. Keratin Azure is a keratinous substrate dyed with Ramazol Brilliant Blue R. The dye is associated with the substrate; the measurement of keratin degradation is expressed by release of the blue colour. If the microbe releases the blue

colour into the medium then this can easily measured using a spectrophotometer (Wainwright, 1982).

2.2.3.2. Preparation of keratin azure solution

Keratin azure was cut into small pieces, 5g keratin azure was added to 300 ml distilled water, put on a shaker overnight and then the blue liquid was added to test media. Keratinolytic assays were achieved via three methods:

- Measurement of clear zone in solid medium-keratin azure (Muhsin *et al*., 1997).
- Release of the blue colour in liquid medium-keratin azure (Letourneau *et al*., 1998).
- Release of sulphur in agricultural soil modified with hoof and horn meal.

2.2.3.3. Measurement of fungal clear zone on solid medium-keratin azure

All fungal isolates were selected for enzyme activity in two types of solid medium Sabouraud Dextrose agar-keratin azure medium (SDA-azure) and Agar–keratin azure medium (A-azure).

SDA-azure medium

The aim of this experiment to see if carbon and nitrogen addition inhibited keratinase production. 48 fungal isolates were cultured on SDA-azure containing extra carbon source (glucose) and nitrogen source (peptone) with keratin azure 5% (v/v). Keratin azure solution was mixed with SDA and the medium was sterilized and poured in plates at a final pH of 5.6. A disc (8mm) of each fungal culture was transferred to plates containing test medium and incubated at 25°C. The growth rate was measured after 2 weeks.

A-azure medium

This experiment aimed to determine growth on keratin azure as the sole source of carbon and nitrogen in the medium. All fungal isolates were cultured in agar keratin azure. 5% (v/v) Keratin azure solution was add to agar bacteriological (Agar No.1.Oxoid). The supplemented medium was sterilized and poured in plates at final pH (7.5). Enzyme activity was expressed by the diameter of clear zone around the fungal colonies. For this test; 8mm disc of each fungal culture was inoculated in plates containing the test medium, incubated at 25 °C for 2 weeks. The clear zones around the colonies were measured. Each isolate that produced visible clear zone on A-azure was selected for keratinolytic assay in liquid medium.

2.2.4. Genomic DNA extraction for isolated fungi

Genomic DNA was isolated from six strains namely; H2, H3, H8, H18 (from hair baited soil), R (from desert) and F22 (from feather) grown in SDB using Norgen Fungi Genomic DNA Isolation Kit (GENEFLOW LIMITED, Labstore, UK) and PowerSoil® DNA isolation kit (MO BIO laboratories) by following procedures.

2.2.4.1. Using (Norgen Fungi Genomic DNA Isolation Kit)

1- Fungi in culture: 50mg of pure fungal culture (wet weight) were grown for three days in Sabouraud dextrose broth medium and transferred to microcentrifuge tubes, centrifuged at 14,000×g for 1 minute to pellet the cells. The supernatant was then poured off carefully.

2- Lysate preparation: the cell pellet was re-suspended. 500 µl of lysis solution was added to the pellet then the cells were re-suspended by gentle vortexing .Optional RNase used by add 10 KUnits of RNase to the suspension and mixed.

3- The mixture was transferred to a provided bead tube and vortex horizontally for 5 minutes on a flat bed vortex by uses any commercial available equipment. The mixture was incubated at 65°C for 10 minutes with mixing 2 or 3 times during incubation.

4- The mixture was transferred into a DNase-free microcentrifuge tube by pipetting and centrifuges it at 14,000 x g for 2 minutes.

5- Carefully, the clean supernatant was transferred into a new DNase-free microcentrifuge tube without disturbing the pellet.

6- Addition of ethanol: equivalent volume of 96%-100% ethanol was added into the mixture and vortex to mix.

7- Addition of binding solution: 300µl of binding solution was added into the mixture and vortex it immediately.

8- Binding nucleic acids to column: the lysate with ethanol (approximately 650µl) was transferred into a column then centrifuged at 6,000×g 1 minute then the supernatant was removed.

9- Column washing: the wash buffer was diluted by absolute ethanol before use. 500 µl of wash solution was added to the column and centrifuged for 1 minute at 6,000×g then discard flow through. The column was centrifuged second time at 6,000×g for 1 minute to remove remaining ethanol. The column was centrifuged at 14,000xg for 2 minutes then discard the collection tube.

10- DNA elution: the column was placed into elution tube to collect DNA. 100 µl of elution Buffer was added into column then centrifuged at 6,000×g for 2 minutes to elute DNA (additional elution may be performed if required).

11- Storage of DNA: the purified DNA was stored at -20°C for a few days and -70°C for long term storage. DNA was run on agarose gel.

2.2.4.2. Using PowerSoil® DNA isolation kit

Genomic DNA was isolated from one strain (H2) using PowerSoil® DNA isolation kit because the previous method was not suitable for this fungus. The fungus was grown in SDB and DNA extracted using PowerSoil® DNA isolation kit by following procedures.

- 500 µl of fungal culture were grown for three days in Sabouraud dextrose broth medium and transferred to the Power Bead Tubes (provided).Then mixed gently.
- 60 µl of **solution C1** was added and inverted many times. Solution C1 must be checked if it is precipitated by heat it at 60°C check.
- The tubes were mixed horizontally for 10 minutes on a flat bed vortex by uses any commercial available equipment.
- The tubes were centrifuged at 10.000× g for 30 seconds at room temperature.
- Using a clean 2ml collection tube, 400-500 µl of the supernatant was transferred. Then 250 µl of **solution C2** was added, mixed for 5 seconds and incubated at 4°C for 5minutes.
- The tubes were centrifuged at room temperature at 10.000× g for 1 minute.
- Up to 600 µl of supernatant were transferred to a clean 2 ml collection tubes.
- 200 µl of **solution C3** were added, briefly mixed and incubated at 4°C for 5 minutes.
- The tubes were centrifuged at 10.000× g for 1minutes at room temperature.
- 750 µl of supernatant (avoiding the pellet) were transferred into a clean 2ml collection tube.
- 1200 µl **solution C4** were added to the supernatant then mixed 5 seconds.
- Approximately 675 µl were loaded into a spin filter and centrifuged at 10.000× g for 1minute at room temperature. The flow through was discarded, additional 675 µl of supernatant was added to the spin filter and centrifuged at 10.000× g for 1 minute at room temperature.

- The remaining supernatant was loaded into the spin filter and centrifuged at 10.000× g for 1 minute.

- 500 µl of **solution C5** was added, centrifuged for 30 seconds at 10.000× g then the flow through was discarded.

- The tubes were centrifuged again at 10.000× g for 1 minute.

- A spin filter was placed in a clean 2ml collection tube. Then 100 µl of **solution C6** was poured in the centre of the white filter membrane.

- The tubes were centrifuged at 10.000× g for 30 seconds at room temperature.

- The spin filter was discarded and the DNA was stored at -20 to -80°C.

2.2.4.3. Agarose gel electrophoresis

DNA fragments were separated by using 1 % agarose gel. These gels were prepared by dissolving 0.5 g of molecular biology grade agarose in 50 ml of 10x TAE buffer. The mixture was heated in a microwave approximately 3 minutes until dissolving the agarose and the solution was cooled. Then, 2.5µl ethidium bromide was added to the mixture before setting the solution in gel tray, and then the gel was poured in the gel rack. After that, the comb was kept at room temperature. The gel was flooded in 10x TAE buffer. Samples were mixed with 2µl loading dye then were added to the wells. 6µl of Hyper Ladder was used in one well in order to find out the size of fragments. The samples were then undergone electrophoresis for 40 min operated at 80V. Digital image was taken using UVitec linked to a digital camera.

2.2.4.4. Polymerase chain reaction (PCR) amplification of fungal genes

After extraction of genomic DNA from unknown fungi, fungal genes were amplified by using polymerase chain reaction (PCR). Fungal universal Oligonucleotide primers (Forward and Reverse) were used for DNA amplification. Primers were designed specifically to a certain region of the proposed DNA to amplify and were obtained from Eurofins (mwg/operone). The fungal gene was amplified with the universal fungal forward primer ITS1 5' TCC GTA GGT GAA CCT GCG G 3' (19) and universal reverse primer ITS4 5' TCC TCC GCT TAT TGA TAT GC 3' (20) (Reeb *et al*., 2010). A typical PCR mixture (50 µl in volume) contained the following components: 28 µl sterile Milli-Q water, 5.0 µl 10× Taq Buffer , 2.5 µl (50 mM) $MgCl_2$, 4.0 µl forward Primer, 4.0 µl Reverse primer, 1.0 µl dNTPs, 5.0 µl genomic DNA and 0.5 µl Taq polymerase. The thermal cycling order for amplification of fungal gene used in this experiment was as follows: initial denature at 94°C for 2 min,

followed by 35 cycles of DNA denaturation at 94°C for 0 sec, primer annealing at 56°C for 10 sec, strand elongation at 72°C for 30 sec. Final elongation at 75°C for 2 min. PCR product (10µl) was mixed with 2 µl loading dye then run on a 1% agarose gel. Hyper ladder (6µl) was run in the gel to confirm the correct sized product.

2.2.4.5. Phylogenetic analysis

The samples were immediately sent to the Medical School Core Genetics Unit for sequencing using ITS1/ITS4 as sequencing primers. Fungal gene sequences were compared in The Basic Local Alignment Search Tool (BLAST). All sequences were corrected by the Finch TV software to identify matches with existing characterized sequences.

2.2.5. The keratinolytic approach used for solid medium

As keratin azure is an expensive substrate, this experiment aimed to investigate fungal keratinolytic activity in different structure of solid medium as an alternative rapid screening method for keratinolytic activity. Briefly, *Trichophyton mentagrophytes*, and the unknown fungus, isolated from desert, and unknown fungus obtained from feathers were tested using agar azure media.13ml melted agar was put in small bottles, it was autoclaved and cooled. Aliquots (5ml) of sterile A-azure and SDA-azure were poured over the agar. The medium was inoculated with 8mm disc from a fungal culture and incubated at 25°C for 2 weeks.

2.2.6. Bacterial keratinolytic assay in solid medium

The following laboratory strains were tested to investigate bacterial keratinase producers: *Bacillus licheniformis, Bacillus stratosphericus, Bacillus pumilus, Bacillus areophilus, Bacillus altitudinis, Bacillus* sp. and *Bacillus thuringiensis* were examined.

2.2.6.1. Bacterial standard suspension

The standard suspension was prepared in phosphate buffer pH 7 and adjusted to $1.5x10^6$ colony forming units (CFU) in order to obtain 0.5 McFarland (0.135 cfu). The turbidity (colonies from each culture mentioned above into a tube containing nine ml of phosphate buffer pH 7) was measured using a spectrophotometer at 600 nm.

2.2.6.2. Measurement of bacterial clear zone on solid medium-keratin azure

The same medium (A-azure) described above was used to detect the appearance of a clearing zone around bacterial colony.100µl from each suspension was poured and spreaded in A- azure plates. The plates were incubated for 48hours at 37°C. Keratinase regions were detected as clear zones against a blue background.

2.2.7. Fungal keratinolytic assay in liquid medium-keratin azure

Three species of fungi produced huge clear zone in A-azure medium. These fungi were selected for enzyme activity in liquid medium. The reaction mixture contained 10% (v/v) keratin azure solution in Erlenmeyer flasks (250 ml) added to SD broth (SDB) (Sigma). SDB consisted of 10g peptone, 40g Dextrose in 1L of dH2O. The mixture was sterilized at final pH 6.06 .The medium was then inoculated with 8mm disc of fungus and incubated on revolving shakers (150 rpm) at 25°C for 7,14, 21 and 28 days. A set of uninoculated flasks was included as control. After the end of incubation period the liquid medium was filtered using Whatman filter paper No. 1. The filter paper with biomass was dried in oven over night at 110°C to a stable weight (Jain *et al*., 2012). The pH was measured during the incubation period using a pH meter. Degradation of keratin azure was determined using the method as described by Letourneau *et al*.,(1998). Keratinolytic activity was determined by measuring the absorbance of keratin azure at 595 nm. The samples after each incubation were filtered and the absorbance of the supernatant was determined at 595 nm.

2.2.8. Bacterial keratinolytic assay in liquid medium-keratin azure

Bacillus licheniformis keratinase was evaluated following growth on LB medium supplemented with azure (LB-azure). LB medium consisted of 10g of tryptone, 10g of NaCl and 5g of yeast extract a 1L of dH2O. 10% (v/v) keratin azure solution was added to LB medium in Erlenmeyer flasks (250 ml) .The medium was sterilized at final pH 7.2. The flasks were inoculated with *Bacillus licheniformis* and incubated on revolving shakers (200 rpm) at 37°C for 12, 24 and 48 hours. The pH was measured during the incubation period using a pH meter. Keratinolytic activity was determined by measuring the absorbance of keratin azure at 595 nm.

2.2.9. Screening of the most active keratinolytic fungi in different media

According to the results (activity of keratinolytic fungi were expressed by the diameter of clear zone and release azure), keratinolytic fungi were tested on two types of solid medium; SDA and potato dextrose agar (PDA) (Sigma), PDA consisting of 4g potato extract, 20g Dextrose an 15g agar in 1L of dH_2O. In addition, two types of liquid medium also were used SDB and potato dextrose broth (PDB) (Sigma). For this test, an 8mm disc from a fungal culture was inoculated on the media. The flasks and the plates were incubated at 25°C for 2, 7 14 days.

2.2.10. Determination of the oxidation of sulphur in agricultural soil amended with hoof and horn meal

The keratinous substrate, hoof and horn meal was used in this experiment. This substrate is commercially produced by heating hoof and horn at high temperature and pressure in order to use it as animal feed and fertilizers (Tapia and Simões, 2010). Agricultural soil (50g was placed in polythene bags and adjusted with 1 g hoof and horn meal (Elixir, Cumbria .UK) then mixed well. Soil without modification was run as a control. The modified soil was incubated in polythene bags, closed with small holes to allow for gas exchange. The bags were then set up in triplicate and incubated for 28 days at 25°C. At zero time, 7, 14, 21 and 28 days the samples were extracted. Soil samples (1g) were shaken with (10ml) of distilled water approximately 15 min at 100 rpm using an orbital shaker. The samples were then filtrated through Whatman No.1 filter paper.

Determination of sulphate

Sulphate was determined as described by (Hesse, 1971). Filtrate (5ml) in 50ml volumetric flask was mixed with (1g) of barium chloride and (2ml) of gum acacia (0.25% w/v). The solution was made up to 25ml with distilled water. The resultant white suspension was measured at 470nm by using spectrophotometer. The concentration of sulphate was then determined by reference to a standard curve (0-100 μg $SO4^{-2}S$ ml^{-1}) prepared from a standard solution of Na_2SO_4 (see Appendix A).

Statistical analysis of data

All observations were presented as Mean ± SE (Standard error). Sigma Plot© (Version12.0) was run to analyze data. $P < 0.05$ was considered as significant. Matching three samples t-test and ANOVA was performed to check whether there were significantly different.

2.3. Results and discussion

2.3.1. Isolation and molecular identification of keratinophylic fungi

By using the Norgen and PowerSoil® DNA isolation kit, six unknown fungi were identified from hair, feather bait and desert soil. They involved H2, H3, H8, H18 (from hair), F22 (from feather) and R (from desert).

2.3.1.1. Isolation of keratinophylic fungi using HBT

Figures 2.1 and 2.2 show that there was good fungal growth in the agricultural soil (pH 7.0) when degreased hair and wool baits were used. The mycelium was clearly visible and the

fungi from soil colonized both baits after 30 days. By using the dilution plate methods, the largest number of isolates was seen to be produced from human hair with the highest total counts (27/48).

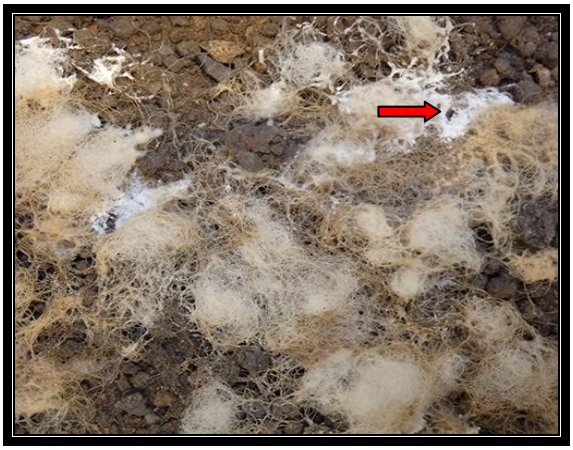


Figure 2.1: HBT using wool as baits.

Figure 2.2: HBT using hair as baits.

The mycelium is indicated by red arrow.

It can clearly be seen that the fungi from soil colonized hair and wool as a source of carbon and nitrogen after one month. This result is in agreement with the findings of Sharma and Rao (2011) who reported that human hair tends to be more highly colonized and degraded than animal hair. As a result, human hair is excellent substrate for use in the study of keratin degradation by fungi. Similarly, Sharma and Sharma (2010) proved that hair fragments are more suitable for fungi than nail baits; however, Saber *et al*., (2009) reported that sheep wool was lowly colonized. Fungal growth showed maximum occurrence on fragments of human hair, which probably indicates their ability to parasitize humans (Singh *et al*., 2009). As a result, many authors have recommended the use of human hair in keratinolysis test because human hair contains no lipids, has few non-keratins and has a high sulphur content which promotes keratinolysis (Kaul and Sumbali, 1999).

2.3.1.2. Phylogenetic identification of unknown fungi

Polymerase chain reaction (PCR) with the help of ITS1 and ITS4 primers, used to amplify fungal genes. Agarose 1% gel was used to check the expected size of the amplified product. Figure 2.3 illustrates the successful PCR products.

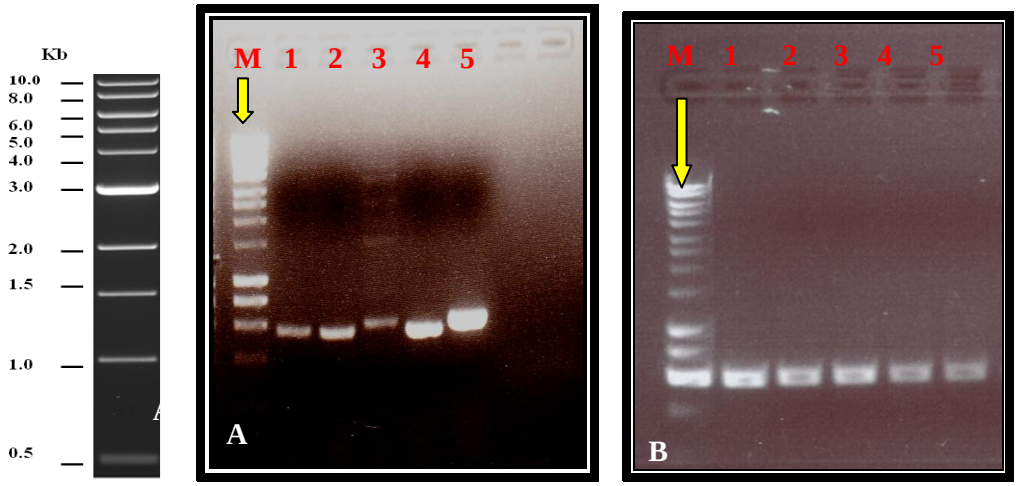


Figure 2.3: Products of fungal genes from PCR. (A) Lane 1: *Acremonium strictum*, Lane 2: *Penicillium verruculosum*, Lane 3: *Mucor hiemalis*, Lane 4: *Aspergillus flavus* and Lane 5: *Mortierella amoeboidea*; (B) Lane 5: *Aspergillus niger*. Yellow arrow (M) indicats the 1-kb DNA ladder.

The amplified product was sequenced and searched against fungal sequences accessible in the BLAST database. The results were used to identify species with high and lower similarity. Table 2.1 shows the species; *Penicillium verruculosum*, *Mucor hiemalis*, *Acremonium strictum*, *Mortierella amoeboidea* , *Aspergillus flavus* and *Aspergillus niger* designed as H3, F22 , R, H18, H8 and H2 respectively.

Table 2.1: The fungal sequence analysis of keratinolytic fungi cultured from hair, feather and desert.

Source of Sample	**Representative sequence**	**Closest matches identification**	**Sequence identity**	**Length of Sequence (bp)**	**NBCI (Accession number)**
Hair	H3	*Penicillium verruculosum*	99%	550	JF682635.1
Feather	F22	*Mucor hiemalis*	98%	799	JQ912672.1
Desert	R	*Acremonium strictum*	99%	504	AM262390.1
hair	H18	*Mortierella amoeboidea*	99%	624	HQ630346.1
	H8	*Aspergillus flavus*	99%	567	JN676112.1
	H2	*Aspergillus niger*	99%	583	GU338398.1

Blast analysis of the fungal sequence of H3 showed similarity of 99% with *Penicillium verruculosum* (Fig.2.4).

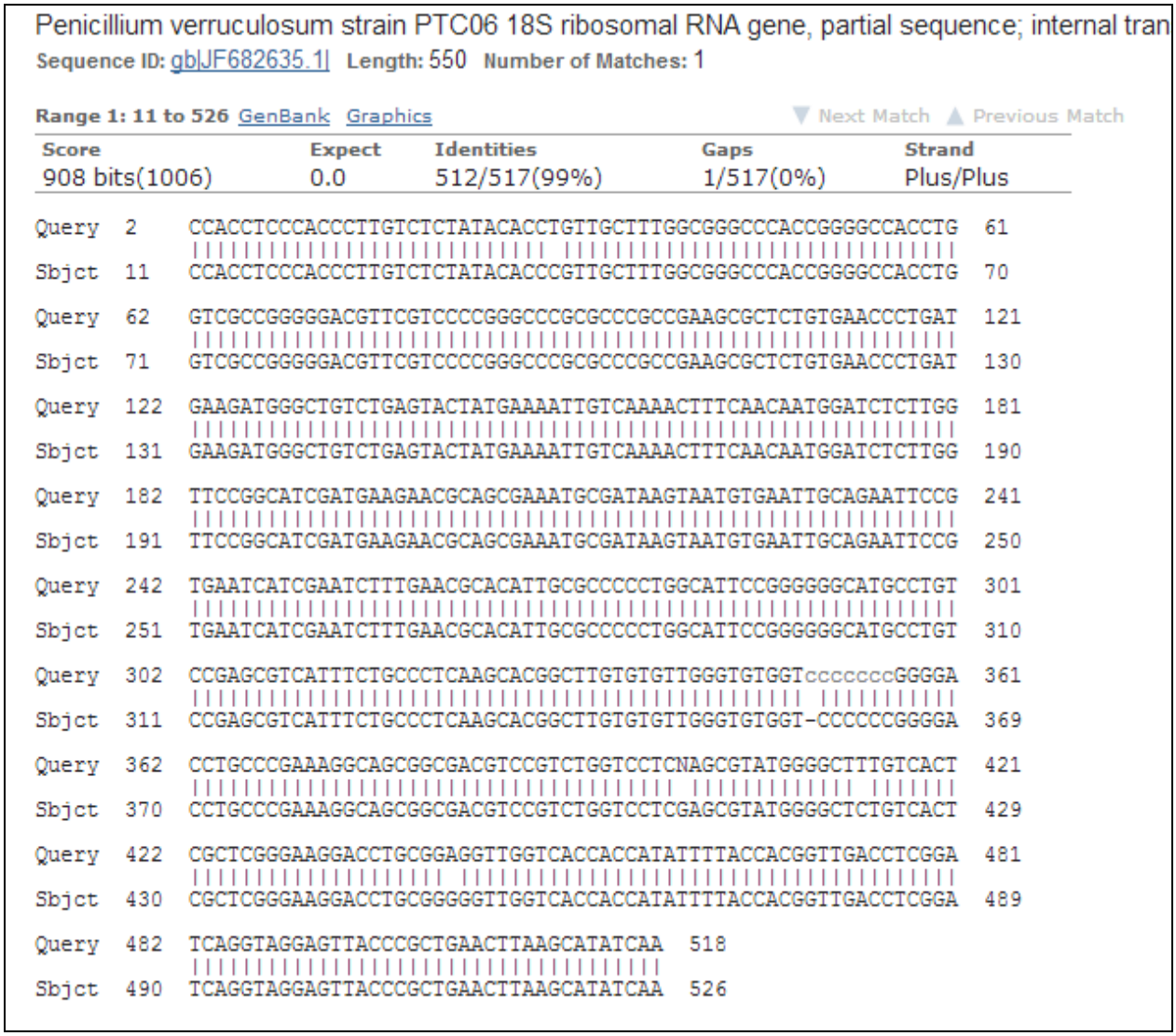


Figure 2.4: Sequence of *Penicillium verruculosum* (achieved after the amplification of 18S rRNA gene) referring to the affiliations through BLAST studies .The "Query" referring to the line when input sequence the "Subject" refers to sequence of line matching.

Sequence analysis of selected isolate showed *Penicillium verruculosum* phylogentically belong to the genus *Penicillium*. In addition, isolate H3 produced phylogentic group with *ascomycetes* and *Penicillium sp.* (Fig.2.5). The dendrogram was organized for its phylogenetic relationship and it detected and confirmed that H3 was *Penicillium verruculosum;* accession number JF682635.1.

Figure 2.5: Phylogenetic tree of H3 (*Penicillium verruculosum*).

This study clearly shows *Penicillium verruculosum* was one of hair isolates (Fig. 2.6 C). In addition, sheep wool produced less isolates (17/48) and 4 isolates were obtained from feathers including *Mucor hiemalis* after one week of growth in SDA (Fig. 2.6 A - B). *Mucor hiemalis* was the most common fungi isolated from feathers. Similar results were reported by Błyskal (2009) and Kim (2003a)**.**

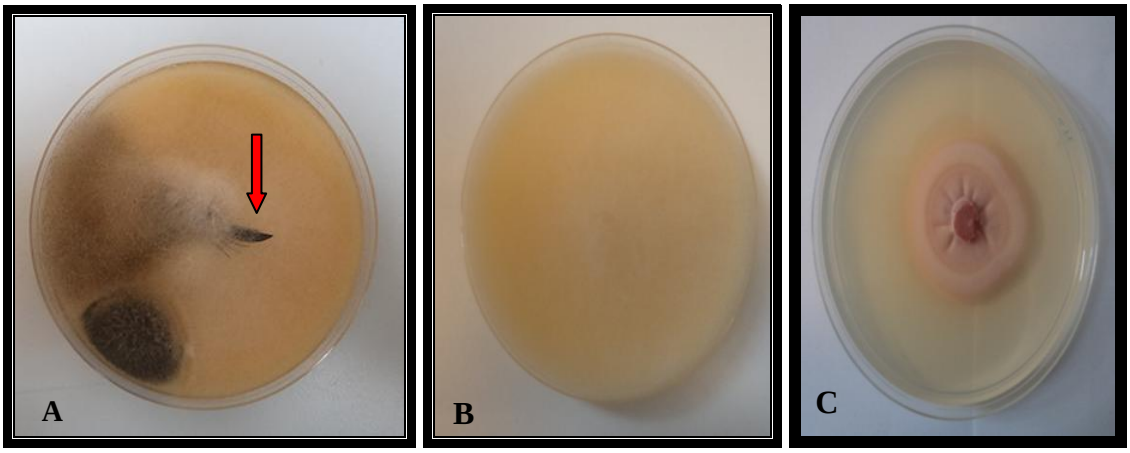


Figure 2.6: Feather (A), *Mucor hiemalis* (B) and *Penicillium verruculosum* (C) in SDA after one week incubation. The feather is indicated by red arrow.

2.3.2. Scanning electron microscopy (SEM)

As we have seen, keratin is degraded by several microorganisms such as bacteria and fungi. Filamentous fungi which can be found in agricultural soil are capable of breaking down keratinous materials such as hair and wool. In this work, hair and wool fragments were used as baits in order to isolate keratinophylic and keratinolytic fungi.

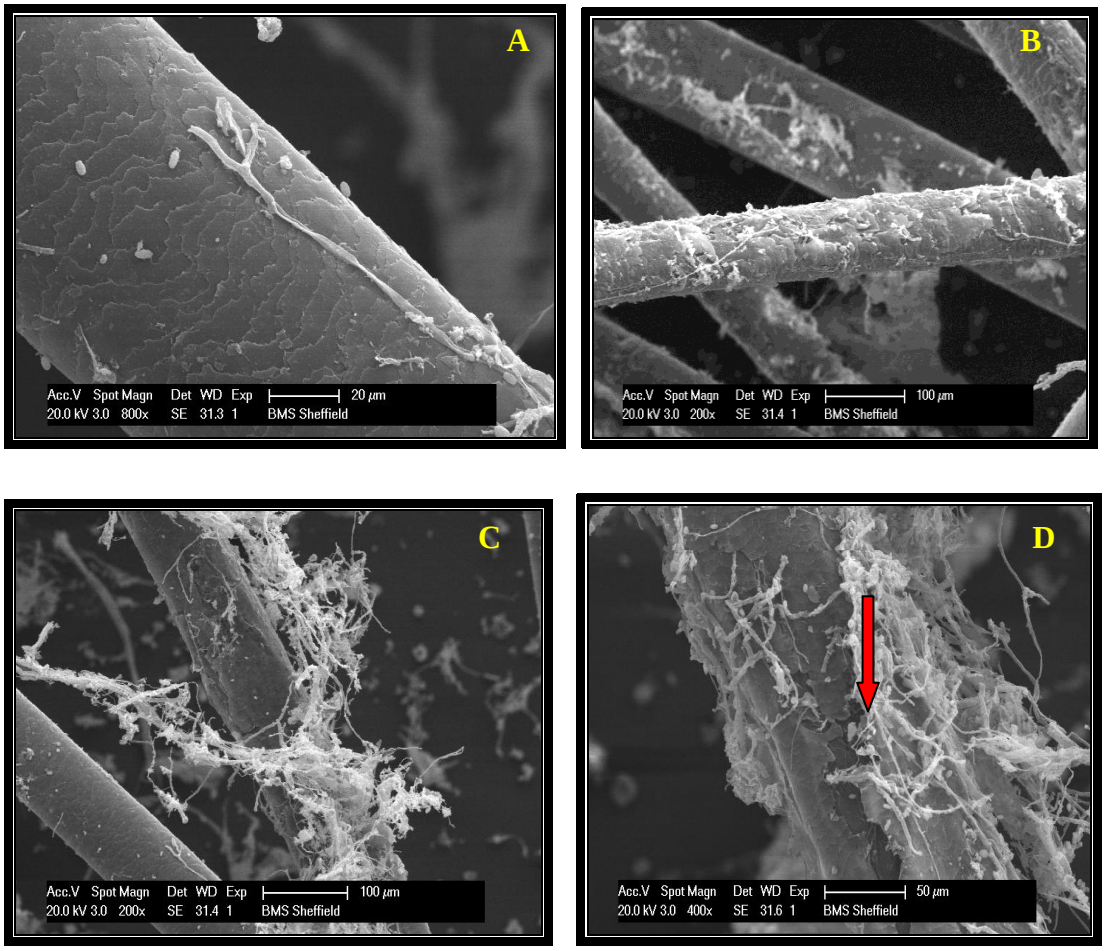


Figure 2.7: Scanning electron micrographs showing colonization of human hair by filamentous fungi after 4 weeks of incubation.

The relationship between fungal structure and cuticular surface of the hair and wool was observed using scanning electron microscopy. This technique was used to record the fungal morphological expression of keratinolysis. Filamentous fungi showed normal hyphae which can be extended to form branches (colonization) and marked growth (Fig. 2.7 A-D). Interestingly, the fungi from soil established a structural relationship with the surface erosion. Surface erosion is the initial stage in hair degradation, as has been reported by many studies (Marchisio, 2000; Błyskal, 2009). In this phase, a gradual degradation from outside of the cuticle (which is covered by several layers) towards the centre can be seen; this results in

decay of the cuticle and the wavy cortex surface (Fig 2.8 F). The current data observations show surface erosion occurring after 4 weeks. English (1969) reported that the cuticle is destroyed slowly and scales remain for 3-4 weeks (partially digested). Figure 2.8 E shows that the keratin molecule was digested.

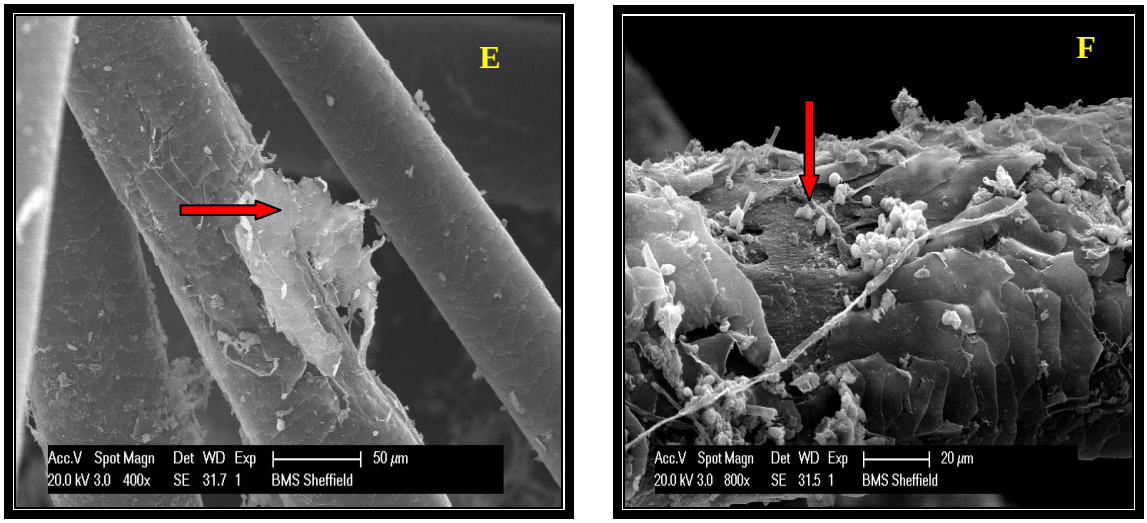


Figure 2.8: Scanning electron micrographs of surface erosion of hair degradation.

Filamentous fungi have the ability to use keratinous material and produce keratinase. Keratinase allows fungi to reach further, internal layers (endocutical). Colonization of sheep wool occurs by the same mechanism, which starts with surface erosion (Fig 2.9 A-B). In addition, the sequence of events was investigated, including digestion of the cuticle. SEM samples from wool showed surface erosion (Fig 2.9 C) and complete degradation of wool (Fig 2.9D). Few studies have reported the sequence of wool degradation. Clearly, surface erosion occurred in hair and wool samples and radial penetration (advanced stage) would also be seen via transmission electron microscopy (TES).

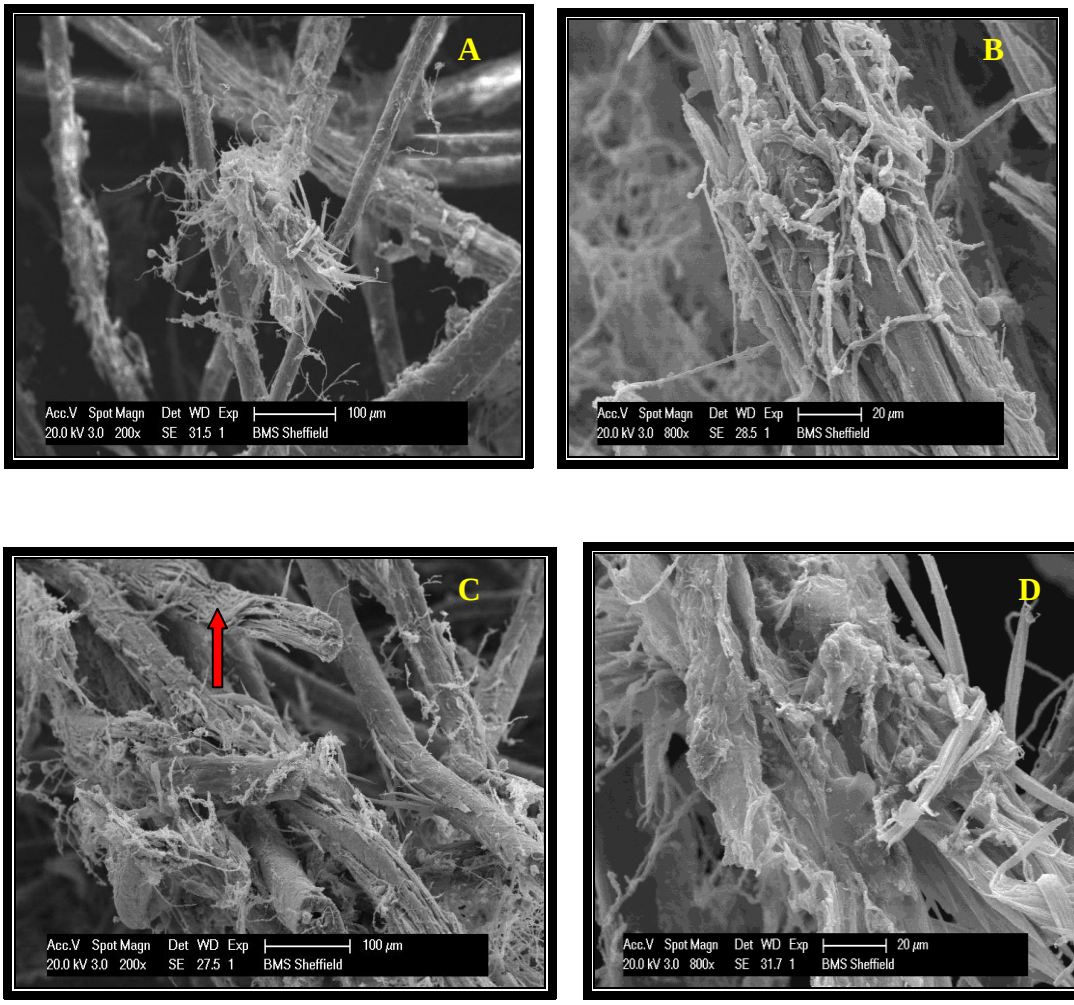


Figure 2.9: Scanning electron micrographs showing colonization of sheep wool by filamentous fungi after 4 weeks of incubation.

2.3.3. Keratinolytic assay on solid media

Two types of solid media; SDA-azure and A-azure were used in order to detect keratinase production in the isolates.

2.3.3.1. Effect of additional carbon and nitrogen sources on keratinase production (SDA-azure medium) in fungal strains

All tested fungi produced good mycelial growth on SDA-azure. The data demonstrated the addition of carbon and nitrogen source has no effect on keratinase production. The growth rate (colony diameter) for *Mucor hiemalis*, *Acremonium strictum*, *Penicillium verruculosum* and *Trichophyton mentagrophytes* was reached to be 77, 45, 71 and 66mm respectively (Fig. 2.10). These data show that carbon and nitrogen sources (glucose and peptone) enhanced biomass growth but not keratinase production (i.e.no clear zone). Keratinase production for all fungi was not seen when SDA-azure was used. Similarly, Santos *et al*., (1996) reported

that with keratin plus glucose and nitrate, the biomass production was substantial, but the keratinolytic activity of *Aspergillus* was significantly lower than that produced in the absence of a carbon and nitrogen source. Also, Jain *et al*., (2012) confirmed that the addition of glucose, maltose, sucrose, sodium nitrate, urea and ammonium inhibited keratinase production in species of *Streptomyces.* The same conclusion was arrived by Mabrouk (2008) who found that the addition of carbon and nitrogen source inhibited keratinase production. However, the results are in disagreement with those of Kim (2003b) who showed that glucose enhanced keratinase production. Generally, the findings reported here show that in the presence of glucose and peptone, mycelia growth was obvious but keratinolytic activity was absent.

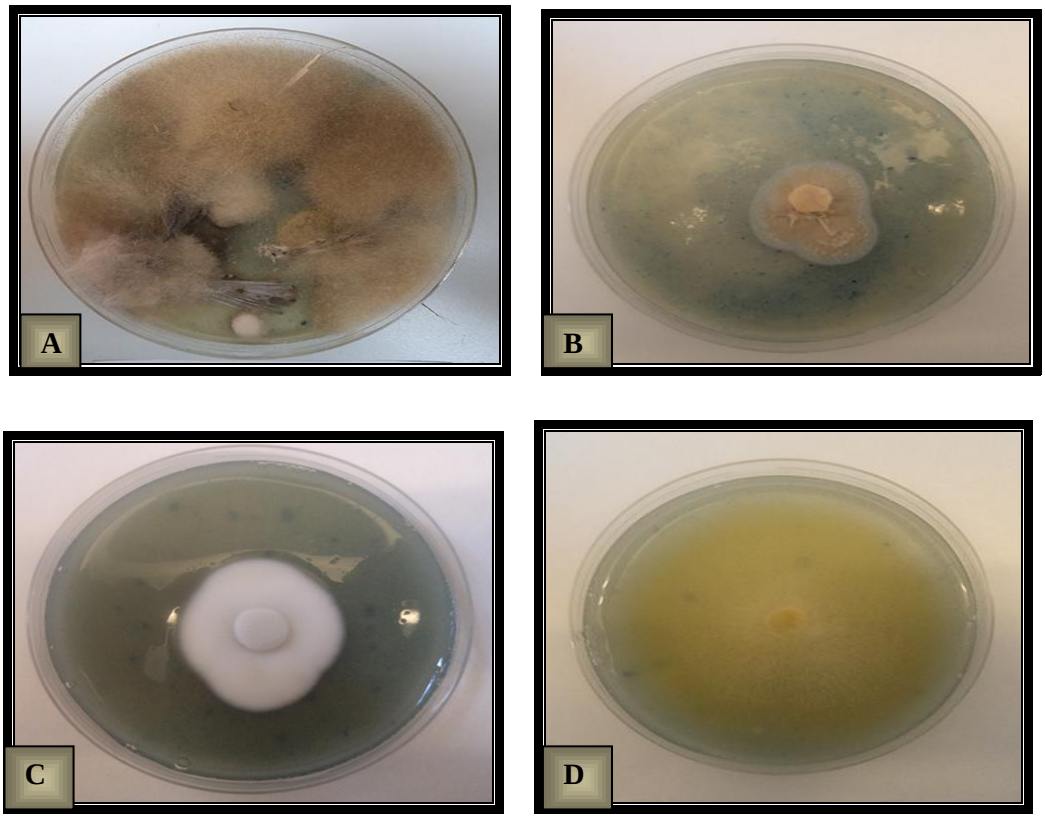


Figure 2.10: The effect of additional carbon and sources on keratinase production after one week. (A) Feather, (B) *Acremonium strictum,* (C) *Trichophyton mentagrophytes.* (D) *Mucor hiemalis.*

2.3.3.2. Effect of absence of carbon and nitrogen source on keratinase production

Most keratinolytic fungi can use keratin as source of carbon and nitrogen. The current data showed that a small number of fungal isolates were able to exhibit enzyme activity (i.e. a

clear zone). The enzyme activity of fungal species is shown in Figure 2.11. Clearly, the isolates obtained from hair (*Penicillium verruculosum)* were more actively keratinolytic than those isolated from soil and desert samples. *Penicillium verruculosum* (a non-dermatophyte) exhibited the highest keratinase activity (59mm) while *Acremonium strictum* (a non-dermatophyte) and *Trichophyton mentagrophytes* (dermatophytes) produced 47,42mm respectively. *Mucor hiemalis* showed weak growth but did not clarify the keratin agar. Obviously, A-azure allowed for the evaluation of keratinase activity by fungal isolates. *Trichophyton mentagrophytes, Acremonium strictum, Penicillium verruculosum, Aspergillus flavus* and *Aspergillus niger* were capable of using keratin as a source of carbon and nitrogen. The obvious explanation for this is that the absence of carbon and nitrogen source motivates the fungus to digest keratin as carbon source leading to increased keratin degradation (Saber *et al*., 2009).

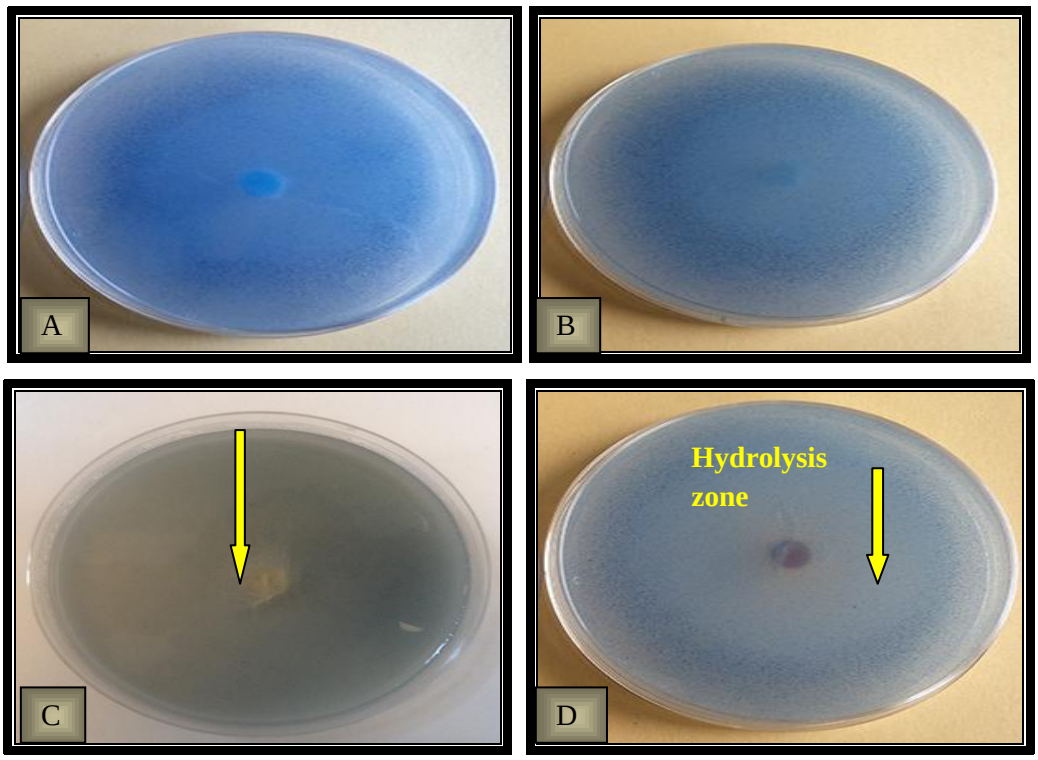


Figure 2.11: The effect of absence of carbon and nitrogen source on keratinase production after 14 days. (A) *Trichophyton mentagrophytes*, (B) *Acremonium strictum*, (C) *Mucor hiemalis* and (D) *Penicillium verruculosum.*

The present data has demonstrated that dermatophytes and non-dermatophytes are both capable of breaking down keratin. These result were confirmed by Muhsin *et al*. (1997) who reported that dermatophytes and non-dermatophytes are both good keratinase producers on test media. Similarly, Rodrigues Marcondes *et al*. (2008) showed that *Acremonium* and

Penicillium were able to grow and produce keratinase in feather medium. However, *Mucor hiemalis* did not create a clear zone reflecting the lack of extracellular keratinase in this fungus. These results are similar to the findings of Filipello Marchisio *et al*., (1991) who reported that *Mucor hiemalis* attacked hair with weak activity. In general then, the presence of added C and N tends to reduce the ability of fungi to breakdown keratin (Veselá and Friedrich, 2009).

2.3.3.3. A new approach for keratinolytic assay

Figure 2.12 gives the result of keratinolytic tests on azure media. It is noticeable that all keratinolytic species showed good growth on SDA-azure medium but there was no clear zone produced in A-azure medium.

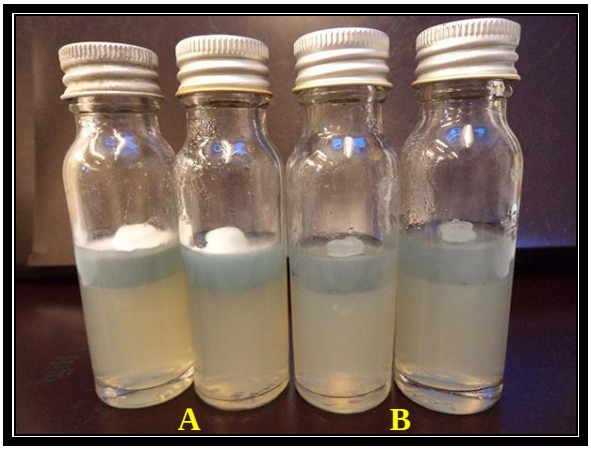


Figure 2.12: *Trichophyton mentagrophytes* grown on tube culture (A) SDA-azure and (B) A-azure after 14 days of incubation.

It is clear that keratinolytic fungi can attack keratin azure in agar plates but not in the tube culture shown above. This approach is therefore of little use for the rapid screening of keratinophylic fungi.

2.3.4. Keratinolytic assay on solid media in bacterial strains (Effect of absence of carbon and nitrogen source on bacterial keratinase production)

Seven bacterial strains isolated from different sources were tested. Among them, three isolates were found to have keratinase activity as assessed by observing clear zones on azure agar plates. *Bacillus licheniformis*, *Bacillus altitudinis* and *Bacillus* sp., isolated from marine sands grew well in solid medium containing keratin as the sole carbon and nitrogen and they decomposed keratin azure. On the other hand, *Bacillus stratosphericus*, *Bacillus pumilus*, *Bacillus areophilus* and *Bacillus thuringiensis* did not produce keratinase (Table 2.2).

Table 2.2: Enzyme activities (EA) of bacterial isolates after 48hours of incubation.

Bacteria	Keratinase production
Bacillus licheniformis strain LZBL-11	Positive
Bacillus stratosphericus strain MCCC 1A04568	Negative
Bacillus pumilus strain XJSL5-8	Negative
Bacillus areophilus strain strain MCCC 1A04568	Negative
Bacillus altitudinis strain SH148	Positive
Bacillus sp. Strain GR-11	Positive
Bacillus thuringiensis strain PTK2G	Negative

The current data demonstrated that small a number of bacteria are able to degrade keratin. Consequently, the hydrolysis of keratin azure was mostly found to be weak among bacterial species. *Bacillus altitudinis* SH148 produced clear zones when grown in keratin azure agar plates. Kumar *et al*., (2011) reported that *B. altitudinis* GVC11 degrades feather-keratin and it can use feather as carbon and nitrogen source. Also, the results showed that clear zones derived from *Bacillus sp*. provided a good indicator for keratinolytic potential. Daroit *et al*., (2009) also showed that *Bacillus sp*. P45 is an excellent keratinase producer on feather meal agar plates. Several studies have confirmed that *Bacillus sp.* is good keratinase producer (Corrêa *et al*., 2010 ; Suntornsuk and Suntornsuk, 2003). Previous experiment also demonstrated that *Bacillus licheniformis* showed keratinase activity and produced clear zones in azure medium. This result in agreement with the findings reported of Lin *et al*., (1992) which indicated that *Bacillus licheniformis* produces clear zones when grown on milk-agarose plates. High activity of keratinase in *Bacillus licheniformis* has been confirmed by several studies (Lal *et al*., 1999; Zerdani *et al*., 2004; Manczinger *et al*., 2003). It can be concluded that the release of keratinase allows bacterial strains to survive when the keratinase is the only available carbon and nitrogen source.

2.3.5. Fungal keratinolytic assay in liquid medium-keratin azure

Under experimental conditions, the maximal hydrolysis rate of keratin-azure was achieved within 28 days at 25°C. The results for 1 to 4 weeks are shown in Figure 2.13. All isolates

showed a rapid response (i.e. dye release at 7 days). Azure release by *Penicillium verruculosum* was higher than that released by *Trichophyton mentagrophytes* and *Acremonium strictum.* This proportion increased after 28 days incubation. However, isolates of known keratinolytic species showed slow keratin degradation at 4 weeks and only produced weak dye release (i.e. *Trichophyton mentagrophytes*) compared to *Penicillium verruculosum* and *Acremonium strictum.*

Significant difference ($P<0.05$) in keratin degradation was observed between all tested fungi. In addition, the results show that non-dermatophytic fungi were able to extract nutrients from the substrate (*Penicillium verruculosum).* However, *Trichophyton mentagrophytes* showed the lowest ability to release azure in test medium. These results is in agreement with those of Scott and Untereiner (2004) who explained that *Trichophyton spp.* was slower to release azure dye between 7 and 28 days of incubation.

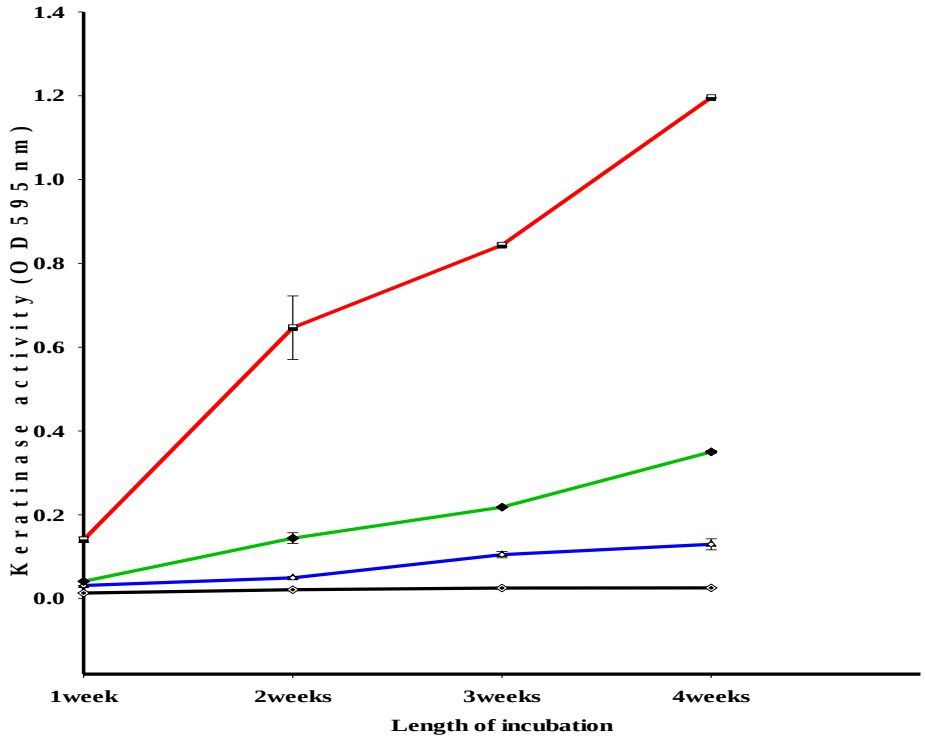


Figure 2.13: Release of azure in SDB-Azure by fungi; *T.mentagrophytes* ( ), *P.verruculosum* ( ), *A.strictum* ( ) and control ( ). Means of triplicates (±) standard error.

2.3.5.1. Biomass

In Figure 2.14 it can be clearly seen that the maximum biomass produced by *Acremonium strictum* occurred after 7 days, growth then decreased gradually in the days following. On the other hand, in biomass production in *Trichophyton mentagrophytes* on SDB-azure gradually increased from 7- 21 days incubation and in keratin production between 7-28 days, after which growth decreased.

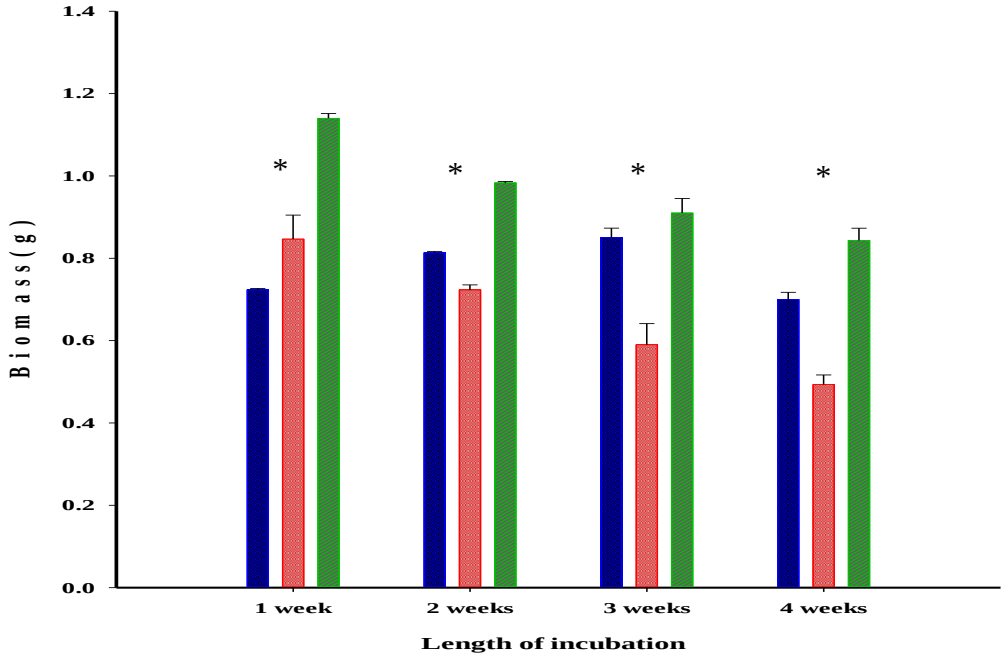


Figure 2.14: Fungal biomass production in SDB-Azure medium.*T. mentagrophytes* (▬), *P. verruclusum* (▬), *A.strictum* (▬). Means of triplicates (±) standard error. *significant difference between *A. strictum, T. mentagrophytes* and *P. verruclusum* .

Significant difference ($P<0.05$) in biomass production was observed in most of the tested fungi. The data reported that the growth was not connected with the degree of release of the dye. These results are similar to the findings by Kim (2003b). For example, *Acremonium strictum* and *Penicillium verruculosum* caused release of dye in the absence of visible growth. As a result, no relationship existed between the enzyme yield and increase of biomass. Similarly, this result is in agreement with the findings of Scott and Untereiner (2004) who reported that *Amauroascus purpureus* caused the release of azure without significant growth.

2.3.5.2. Change in pH

Change in pH of medium was noted after 1 week. The experimental results show that the degradation of keratin azure was accompanied by alkalization of the medium. A slight change

in pH was measured in the control while the fungal samples recorded a significant change in pH. A maximum pH value was 8.8 was seen when *T. mentagrophytes* grew in liquid medium. However, *Acremonium strictum* caused a decrease in the pH level of the medium (Fig. 2.15) in week 1and 2 after that pH increased dramatically.

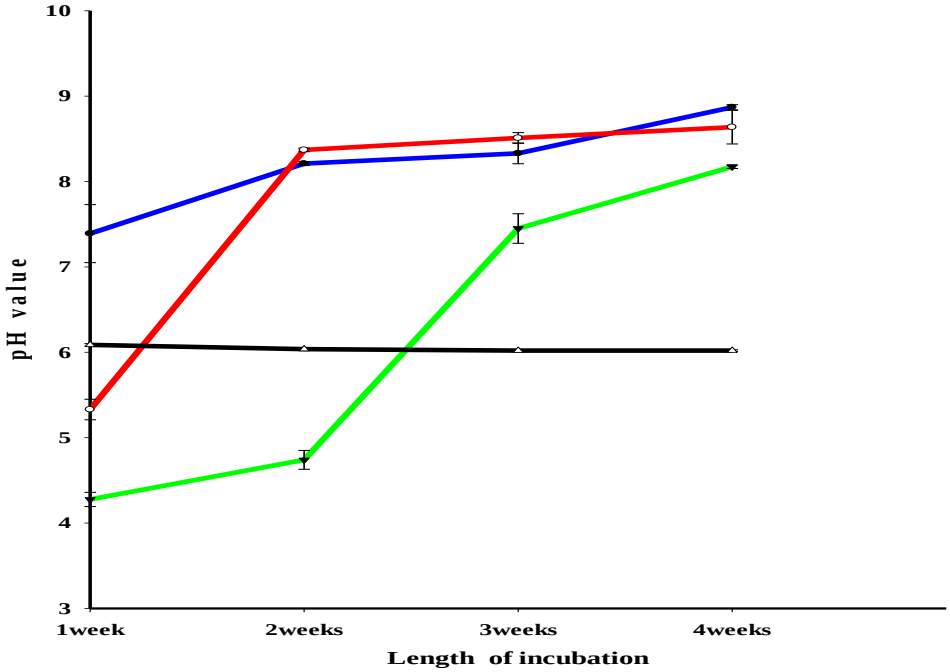


Figure 2.15: pH values of fungal isolates during incubation period. T. *Mentagrophytes* (), *P.verruculosum* (), *A.strictum* ( ) and control (). Means of triplicates (±) standard error.

A significant difference (P<0.05) in pH range was observed in all tested fungi. The alkalinisation by fungi was highly marked in medium contain keratin azure. This may reflect that keratinolytic fungi often alkalinize culture media during growth (Muhsin and Hadi, 2002). Interestingly, this correlation is related to the release of ammonia which raises the alkalinity of the medium. Jain *et al*., (2012) have shown that a change in pH (deamination) toward alkalinity allows the substrate to create an environment for the sulphitolysis and proteolysis. The results also demonstrated that the fungi which have strong keratinolytic ability increased alkalinity more than those that were less keratinolytic (Kim, 2003a). The present data confirms that there is an association between the degree of alkalinisation and the amount of keratin degraded. These results are similar to those reported by Kaul and Sumbali (1999), who confirmed strong keratinolytic activity makes growth medium more alkaline.

However, a decrease of pH in the medium recorded in *Acremonium strictum* and *Penicillium verruculosum* may be associated to the typical fungal activity that generates acidification as a consequence of the use of C and N compounds (Elíades *et al*., 2010). In general, however, change in pH of the media is considered as a sensitive indicator of keratinolysis (Rajak *et al*., 1992).

2.3.5.3. Screening of the most active keratinolytic fungi using a range of media

The present data demonstrated that *Trichophyton mentagrophytes*, *Penicillium verruculosum* and *Acremonium strictum* degraded keratin on solid media and liquid media. However, *Penicillium verruculosum* gave a different response in solid and liquid medium; in addition, this fungus secreted a red pigment.

2.3.5.4. Effect of the medium on secretion of red pigment from *Penicillium verruculosum*

The result shows *Penicillium verruculosum* produced interesting "dart-board" colonies which varied depending on the medium used (Fig. 2.16) and also secreted a red pigment (Fig. 2.17). In addition, the pigment was also formed in broth cultures.

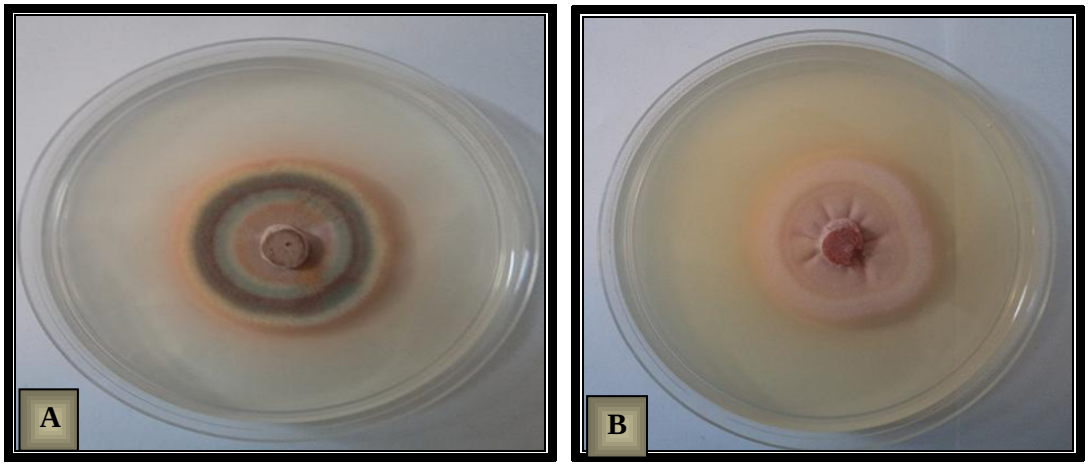


Figure 2.16: Effect of medium on *Penicillium verruculosum* after one week.
(A)PDA medium and (B) SDA medium.

2.3.5.5. Effect of incubation period on secretion of red pigment from *Penicillium verruculosum*

Figure 2.17 shows that the red pigment is visible after 14 days incubation on solid medium; however, it was observed after 2 days incubation in liquid SDB.

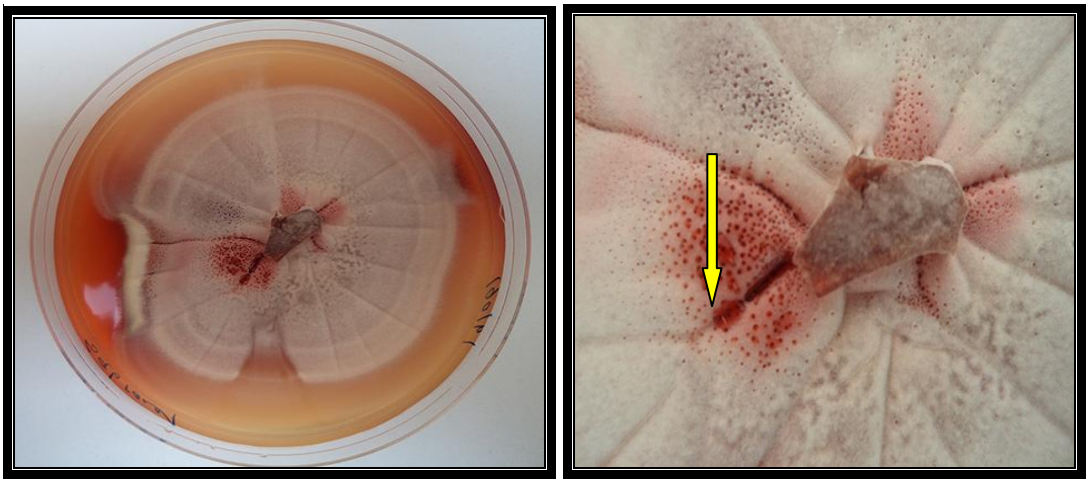


Figure 2.17: Incubation of *Penicillium verruculosum* after 14 days.

Undoubtedly, two conditions affected red pigment secretion from *Penicillium verruculosum.* The results showed that the use of medium containing peptone and glucose influenced red pigment production. In addition, the pigment was released after 48 hours in SDB. The result are similar to those of Nasuno and Asai (1961) who examined *Penicillium sp.* using SDB and found that a red pigment was secreted after 48 hours incubation in broth medium.

2.3.6. Bacterial keratinolytic assay in liquid medium-keratin azure

Bacillus licheniformis strain LZBL-11 was studied to determine keratinase activity against keratin azure. In addition, it was selected using keratin azure digestion assay. Figure 2.18 illustrates the results of keratinolytic activity derived from *B. licheniformis.* There was a considerable increase in keratinase through 96 hours. The amount of keratinase increased gradually and the maximum keratinase activity was found at 96 hours. On the other hand, the control remained stable during incubation period. Lin *et al*., (1995) reported that the maximum values of keratinase from *B. licheniformis* were observed between 48 and 60 hours growth. Also, Tiwary and Gupta (2010) reported that the maximum keratinase derived from *B. licheniformis* was achieved after 36 hours using keratin azure at 595nm. A significant difference ($P<0.05$) in keratinase production was observed through 24-96 hours of bacterial growth. Current data confirmed that *B. licheniformis* has high keratinase activity and it was able to release azo-dye, measured at 595 nm. Many studies confirm that *B. licheniformis* is considered to be the highest keratinase producer among *Bacillus* species (Sivakumar *et al*., 2012; Korkmaz *et al*., 2004; Manczinger *et al*., 2003).

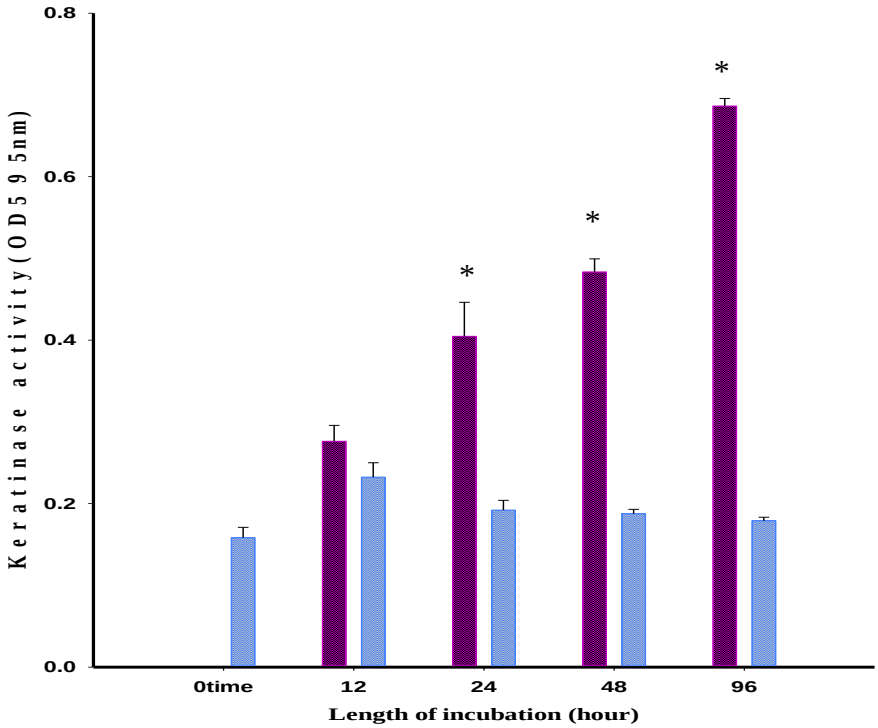


Figure 2.18: Keratinase activity produced by *B. licheniformis* in LB medium supplemented with keratin azure. *B.licheniformis* ( ) and control ( ). Means of triplicates (±) standard error. * significant difference from control value, $P < 0.05$.

2.3.6.1. pH

Figure 2.19 shows to the pH range seen during keratin azure digestion. There was a gradual increase in pH value through incubation period. The maximum pH for keratinase production was 9.7. The pH of the test medium increased from 7.2 to 9.7 during the 96 hours of incubation. The result shows that *B. licheniformis* was capable of keratinase production in alkaline conditions. Significant difference ($P<0.05$) in pH was observed during 12-96 hours. Keratinolytic enzymes have been reported as active enzymes at alkaline pH values (Bernal *et al*., 2003). Additionally, other studies confirm that keratinase derived from *Bacillus* species might be classified as an alkaline enzyme, which is very active under basic or neutral conditions (Suntornsuk and Suntornsuk, 2003; Kumar *et al*., 2011).

2.3.6.2. Biomass production

LB medium supplemented with keratin azure affected growth and keratin production by *B. licheniformis*. The results given in Figure 2.20 show that *B. licheniformis* grew well on modified LB medium. Moreover, there was a significant growth of *B. licheniformis* at 96 hours and good growth under alkaline conditions. However, there was a decrease in growth

after 24 and 48 hours. It can be clearly seen that the lowest growth was shown after 24 hours. LB medium supplemented with azure enhanced the growth and keratinase production.

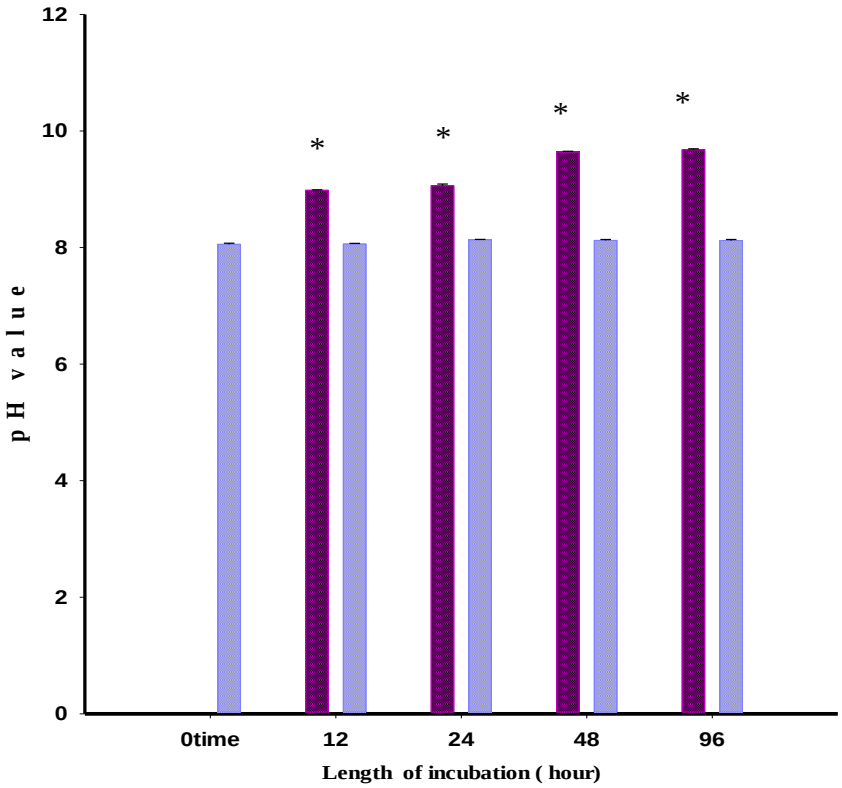


Figure 2.19: pH values produced by *B. licheniformis* in LB medium supplemented with keratin azure during keratinase production. *B. licheniformis* (▬) and control (▬). Means of triplicates (±) standard error. * significant difference from control value, $P < 0.05$.

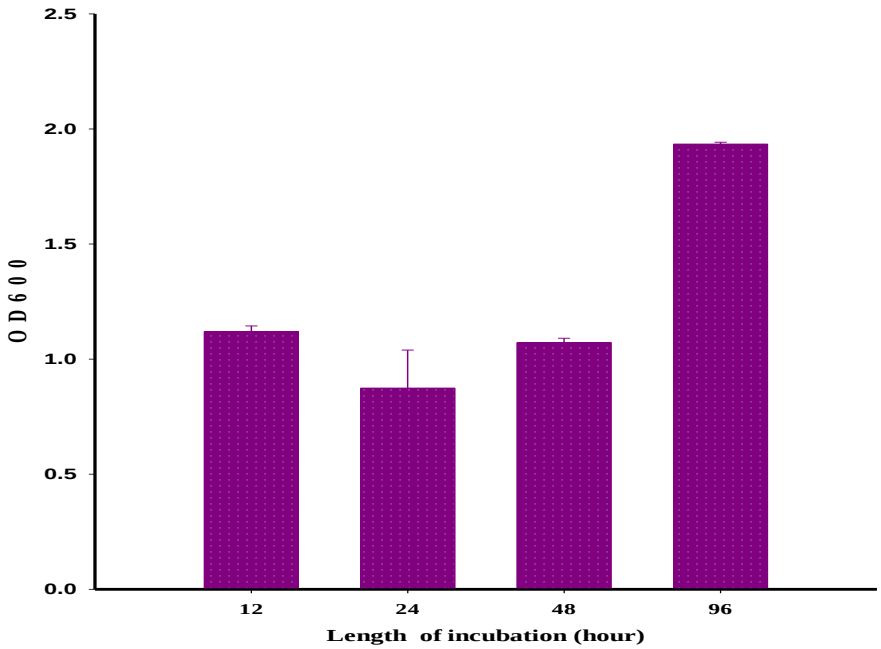


Figure 2.20: The growth of *B. licheniformis* in LB medium supplemented with keratin azure. Means of triplicates (±) standard error.

2.3.7. Determination of the oxidation of sulphur in agricultural soil amended with a keratinous substrate

The release of large amounts of sulphur in the soil is associated with keratin degradation (Rajak *et al*., 1992). Figure 2.21 shows the amount of sulphur produced in the agricultural soil amended with hoof and horn from 1 to 4 weeks incubation. The results show that microbial S-oxidation of sulphur increased over the 4 week incubation period (treatment). It can be clearly seen that there was a significant increase between treatment and control throughout the length of incubation. Moreover, oxidation of sulphur reached a peak of week 3. A Significant differences were observed over the entire period except week 2. A decline in the amount of sulphate in the last phase of the experiment might be result of adaptation of microorganisms such as fungi to use of sulphate as a sulphur source. Sulphitolysis may be refer to the extremely high content of sulphur in hoof and horn (Rajak *et al*., 1992).

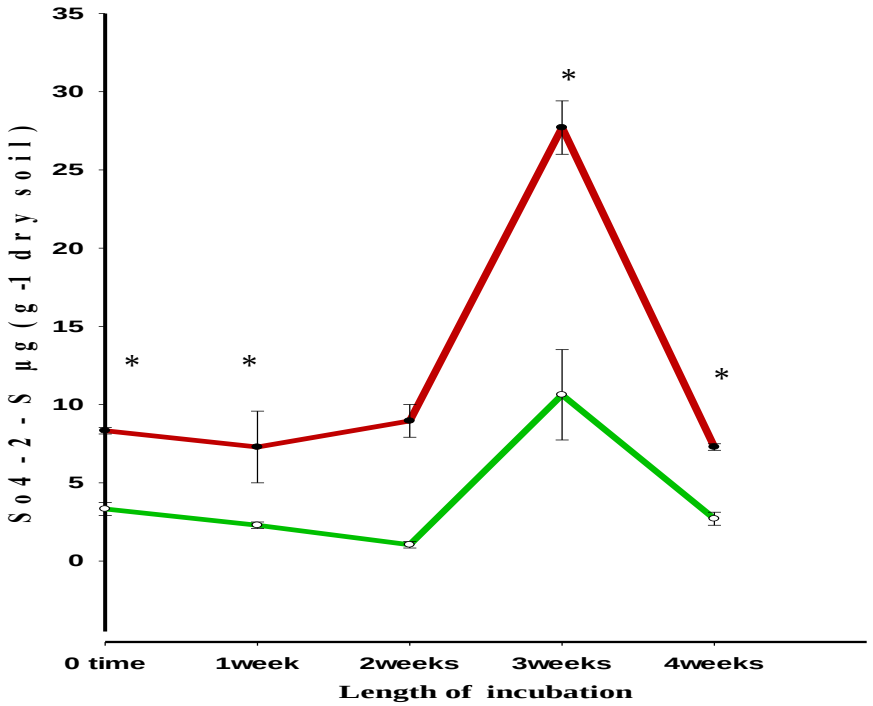


Figure 2.21: Sulphate production from oxidation of elemental sulphur in agricultural soil adjusted with hoof and horn meal. Treatment (—●—) and control (—○—). Means of triplicates (±) standard error. * significant difference from control value, $P < 0.05$.

CHAPTER 3

CHAPTER 3
STUDIES ON MICROBIAL DEGRADATION OF PECTIN

3.1. Introduction

Pectin is a complex polysaccharide found in plants (cell wall and middle lamella); they consist of homogalacturon (HG) which might be methyl esterified or de-esterified to generate co-polymer structure. Also, the HG backbone may be attached with neutral sugars in the side of chains generating the rhamnogalacturon I (RGI) region, or rhamnogalacturon II (RGII) (Wicker *et al*., 2014).

3.1.1.Pectin substances and pectinases

Pectins are found in fruits and vegetables such as the peel of orange and lemons and the pulp of tomato and pineapple. In these substances, D-galacturonic acid units are combined via α-1, 4- glycosidic bonds and the carboxyl groups are esterified with methanol. In addition, rhamnose units can be inserted into the backbone and linked with sugars such as arabinan, galactan or arabinogalactan. As a result, several forms of pectins are found in plant cells. Pectin-rich substances must be broken down enzymatically by pectinases (Gummadi and Panda, 2003).

3.1.2. Classification of pectinases

Pectins are hydrolyzed by pectinases which are one of the most widely distributed of enzymes. Several types of pectinases can be found, including protopectinases, polygalacturonases (PG), pectin lyases (PL) and pectin esterases (PE). Polygalacturonases (PG) are considered to be the richest pectinases; they hydrolyze the main chain using water. Pectin lyase (PL) cleaves the chain by trans-elimination while pectinesterase (PE) promotes de-esterifying of the methyl ester linkages of the pectin backbone and is responsible for the release pectins and methanol. Pectinolytic enzymes have wide application in many sectors such as fruit juice extraction, scouring of cotton, waste water treatment and vegetable oil extraction (Jayani *et al*., 2005).

Pectinases are classified into two groups: pectinesterases (PE) and depolymerases. Pectinesterases enhance the de-esterification of methyl ester bonds of the galacturonic acid component of pectin substrates and release methanol and acidic pectins. While, depolymerases (include polygalacturonase (PG) (endo and exo), pectin lyase (PL), polymethyl galacturonase (PMG) breakdown glycosidic linkages between the two non-methylated acid residues. Endo-PG promotes the random hydrolysis of a-1, 4-glycosidic

linkages. Endo-PGs could be acidic endo-PGs (they are used in wine clarification and in feed additives) or alkaline endo-PGs which can be used in paper industry, pectin wastewater treatment and coffee fermentation (Yuan *et al*., 2011).

3.1.3. Microbial pectinases properties

Many microorganisms produce multiple types of pectinases according to pectin substrates and mode of action on the pectin polymer (Latif and Sohail, 2012). Fungi are the main producers of pectinases; notably *Aspergillus spp*. and *Penicillium spp*. (Yuan *et al*., 2011). Fungi such as *Aspergillus niger, Trichoderma harzianum,* and *Rhizopus* are useful because 90% of the fungal pectinase they contain can be extracted into the medium. There is one limitation to use these fungi, namely they grow slowly compared to bacteria. As a result, bacteria might be preferred for the production of polygalacturonase to meet the industrial demands from these enzymes (Latif and Sohail, 2012). In most biotechnological applications, bacterial pectinase are mainly produced by *Bacillus* and *cocci* species such as *B.licheniformis* and *Staphylococcus aureus.* Approximately half of the pectinase used in this way originates from *Bacillus* and *cocci* strains (Venkata Naga raju and Divakar, 2013).

The aim of the work discussed in the Chapter was:

(i) Screen for pectinolytic fungi and bacteria.

(ii) To assess the ability of bacterial and fungal strains to degrade pectin molecules and identify products (NH_4 and NO_3) of degradation.

(iii) Evaluate the antibacterial effects of pectin against strains causing wound infection.

3.2. Material and Methods

To date various methods have been developed and introduced to determine the activity of pectin-degrading enzymes. In this study, the medium used was pectinase screening agar medium (PSAM) prepared according to the procedure of Hankin and Anagnostakis (1975), with small modifications.

3.2.1. Qualitative assay for pectinolytic microorganisms

Pectinase screening agar medium was divided into two media based on pH. One advantage of this method it allows detecting two enzymes; pectate lyase (PL) and polygalacturonase (PG). The medium contained; 500 ml of mineral salts solution, 1 g of yeast extract, 15 g of agar, 5 g of apple pectin (Sigma), and 500 ml of distilled water. Detection of pectate lyase production [pectate transeliminase] was assessed at pH 7.0. The same medium

at pH 5.0 was used to detect polygalacturonase activity [pectin depolymerise, pectinase]. The mineral salts solution contained; $(NH_4)_2\ SO_4$, 2g ; KH_2PO_4, 4g ; Na_2HPO_4, 6g; $FeSO_4.7H_2O$, 0.2 g; $CaCl_2$, 1 mg; H_3BO_3, 10 µg; $MnSO_4$, 10 µg; $ZnSO_4$, 70 µg; $CuSO_4$, 50 µg in 1L distilled water.

3.2.1.1. Screening for pectinolytic fungi by plate assay

Ten fungal isolates were tested using PSAM medium: *Trichophyton mentagrophytes, Aspergillius oryza, Alternaria tenuissima, Pencillium daleae* (laboratory strains), *Mucor hiemails* (isolated from feather), *Acremonium strictum* (isolated from desert), *Penicillium verrclusium, Aspergillius niger, Aspergillius flavus* and *Motriella amobia* (isolated from hair). A disc (8mm) of each fungal culture was inoculated on the plates containing both test media, incubated at 25 °C for 2 days. The presence of pectin lyase activity and polygalacturonase activity were detected by gently flooding the plates with a solution of iodine-potassium iodide containing (1.0 g iodine, 5.0 g potassium iodide and 330 ml distilled water). This reagent was used to detect clearing zones against a dark background (Soares *et al*., 1999).

3.2.1.2. Screening for pectinolytic bacteria by plate assay

Bacterial samples were tested for pectinase production and pectin lyase as previously reported using the same medium in fungi. *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli (E.coli), Cupriavidus necator, Bacillus megaterium, Rhizobium sp*.and two unknown bacteria B1 and B2 (laboratory strains) were qualitatively screened for the presence of pectin degrading enzymes using suitable dyes. The isolates were used as a standard suspension. Each suspension adjusted to $1.5x10^6$ colony forming units (CFU) which equals 0.5 McFarland. The experiment was run using the isolated colonies into a tube containing 9 ml of phosphate buffer pH 7. Turbidity was measured using spectrophotometer (600 nm) and 0.1 ml was taken from each suspension and then poured and spread onto medium plates. The plates were incubated 48 hours at 37°C. Plates were flooded with the dye solution. The plates were left at room temperature for approximately 10 min. Pectin lyses regions were detected as light clearing zones against a dark black background.

3.2.2. Quantitative assay of pectinolytic bacteria

The isolates were selected on the basis of the positivity of pectinase production during qualitative assay. The isolates showed the largest clear zones were selected for biochemical tests. Quantitative enzyme estimation was done using the dinitrosalicylic acid (DNSA)

method described by Kashyap *et al.,* (2000). *Bacillus megaterium* was chosen and tested for growth and pectin decomposition on two different media: Luria- Bertani (LB) and yeast extract (YE) supplemented with apple pectin (0.25% w/v) as carbon source. LB medium consisted of 10 g of tryptone, 10 g of NaCl and 5 g of yeast extract and 2.5 g of apple pectin in 1L of dH2O at pH 7.2. The composition of YEP medium is: yeast extract (YE), 10.0 g; apple pectin, 2.5 g in 1L of dH2O at pH 7.2. The media were sterilised by autoclaving at 121°C for 15 minutes. The bacteria were grown in two media (50 ml in 250 ml Erlenmeyer flasks) for 12, 24, 36 and 60 hours at 37°C on an orbital shaker (150 rpm) then pectinase activity, pH and cell growth (OD 600 nm) were measured.

3.2.2.1. Pectinase activity

The methods described by Miller (1959) were used for the estimation of pectinase production. Degradation of pectin was determined using the method described by Kashyap *et al.,* (2000).The experiment was run using dinitrosalicylate reagent contained 0.63 % dinitrosalicylic acid, 18.2% sodium potassium tartrate (Rochelle salts), 0.50% phenol, 0.50 % sodium bisulfite, and 2.14 % sodium hydroxide. The reaction mixture involved 100 µl of supernatant, 100 µl of substrate1.0% (w/v) (apple pectin). The mixture was incubated at 40 °C for 10 min. 400 µl of DNSA reagent was added to the mixture and boiled for 15 min. 4.4 ml deionized water was added to the mixture and the yellow colour was measured at 530 nm. Pectinase activity was then determined by reference to a standard curve of galacturonic acid prepared from a standard solution of D-(+) - Galacturonic acid monohydrate (Sigma) (see Appendix A).

3.2.2.2. Effect of salts supplement on pectinase production

In order to optimize *B.megaterium* growth conditions, salts included Mg_2SO_4. $7H_2O$ and $CaCl_2$ were added to LB and YEP media then pectinase activity was assayed using the same methods described above. The percentage of salts as following:

- **YEPC medium:** YEP+$CaCl_2$ (0.05% w/v).
- **YEPM medium:** YEP+ Mg_2SO_4. $7H_2O$ (0.05% w/v).
- **LBPC medium:** LBP+ $CaCl_2$ (0.05% w/v).
- **LBPM medium:** LBP+ Mg_2SO_4. $7H_2O$ (0.05% w/v).

3.2.3. Quantitative assay for pectinolytic fungi

Aspergillius oryza was selected for quantitative enzyme estimation using the medium described by Wang *et al.,* (1997). For qualitative assay, the liquid medium was composed of

2 g of L-asparagine (Sigma), 2.5 g of $Mg_2SO_4.7H_2O$, 2.5 g of KH_2SO_4, and 5 g of apple pectin in 1 liter of deionized water. The mixture sterilized at final pH 5.0. The medium was inoculated with a 8mm fungal disc (50 ml in 250 ml Erlenmeyer flasks) and incubated on a shaker (120 rpm) at 25°C for 3,5,7 days. A set of uninoculated flasks were run as controls. After the end of incubation period, the liquid medium was filtered using Whatman filter paper No. 1. The filter paper with fungal biomass was dried in oven overnight at 110°C to obtain a constant weight (Jain *et al*., 2012). The pH was measured during the incubation period using a pH meter. Degradation of pectin was determined using the method described by Kashyap *et al* (2000).

3.2.4. Chemical analysis of degraded pectin (pectin, esterified potassium salt, from citrus fruit)

Chemical analysis was aimed to detect some minerals from decaying pectin. To demonstrate the potential of this approach and its suitability for the application, nitrate and ammonium production from soil amended with citrus pectin is investigated.

3.2.4.1. Determination of nitrate in agricultural soil amended with citrus pectin

All samples of agricultural soil (50g) were placed in polythene bags and amended with 0.5g pectin, esterified potassium salt, from citrus fruit (Sigma) and mixed thoroughly. A control was set-up lacking added pectin. The modified soils were incubated in polythene bags closed with a small hole to allow for gas exchange. The bags were set up in triplicate and incubated for 28 days at 25°C. At zero time and at 7, 14, 21 and 28 day intervals samples were extracted. After incubation (1g) soils were then placed into screw capped glass bottle with (10 ml) deionised water used to extract nitrate; after shaking for 15min at 100 rpm on an orbital shaker, the samples were filtrated through Whatman No.1 filter paper.

Nitrate Determination

Nitrate was determined using the method of Sims and Jackson (1971). Chromotropic acid (CTA) reagent (7ml) was mixed with 3ml of filtrate the mixture was cooled in cool water and incubated at 40°C in water bath for 45 minutes; the yellow colour CTA-NO_3 formed was measured at 410 nm in a spectrophotometer and the concentration of nitrate was determined by reference to a standard curve (0-100 NO_3-N ml-1) prepared from standard solution of $NaNO_3$.

Reagents: Chromotropic acid

1- Stock solution

1.84 g of Chromotropic acid $C_{10}H_6O_8S_2Na_2$ was dissolved in 1 litre of sulphuric acid H_2SO_4 .The solution was stored at 4°C.

Working solution

Stock solution (100 ml) was diluted in 990 ml of concentrated H_2SO_4 and then added 10 ml concentrated HCl. The solution was stored at 4°C for several weeks.

3.2.4.2. Determination of ammonium in agricultural soil amended with citrus pectin

Soil (50g) was placed in polythene bags and amended with (0.5g pectin) and mixed thoroughly. A control was set-up lacking without added pectin. The modified soil was incubated in polythene bags which were closed with a small hole to allow for gas exchange. The bags were set up in triplicate and incubated for 28 days at 25°C. At zero time and at 7, 14, 21 and 28 days, samples were extracted. Ammonium was extracted from the soil with a solution of KCl (150 g KCl / 1000 water) in the ratio: (1g) soil: (10ml) KCl. The soil was shaken for 30min at 100 rpm on an orbital shaker then filtered through filter paper Whatman No.1.

Determination of ammonium

Filtrate (2ml) was added to (1ml) of EDTA (6% w/v), (7ml) of distilled water, (5ml) of phenolate reagent and (3ml) of sodium hypochlorite solution (10%v/v). The reaction mixture was mixed thoroughly and incubated at 25°C for 20min in the dark. The volume was made up to 50 ml and mixed and the concentration of the indophenol-blue ammonium complex was measured at 630 nm using a spectrophotometer (Wainwright and Pugh, 1973). The concentration of ammonium intensity was then determined by reference to standard curve (0-50µg NH_4-N ml-1) prepared from a standard solution of ammonium sulphate $(NH_4)_2SO_4$.

Reagents

1) Ethylenediaminetetraacetic acid (EDTA):

60 g EDTA was dissolved in 900 ml of distilled water then diluted to1L.

2) Phenol solution:

62.5 g Phenol was dissolved in ethanol (25 ml) and adding acetone (18.5ml) to made up to 100 ml. The phenol solution should be stored in the dark at 4°C.

3) Phenolate reagent: 20 ml of phenol solution was mixed with (20 ml) hydroxide sodium (27% NaOH w/v) and diluting to 100 ml. The reagent was prepared fresh daily.

3.2.5. Antimicrobial activity of pectin

This experiment was designed to determine the effect of pectin on bacteria in relation to controlling wound infection. The following bacteria were used: *Pseudomonas aeruginosa, Staphylococcus aureus, S. aureus* MRSA3 and *Escherichia coli* (*E.coli*). Pectin, esterified potassium salt, from citrus fruit (Sigma) was dissolved in sterile distilled water. The concentration of the solution of pectin was 5% (w/v). The solution was stored at 4°C as stock. Phosphate buffer suspension of all strains, equivalent to 0.5 McFarland standard containing $1.5x10^6$ colony forming units (CFU). Five wells were made in each nutrient agar plate. A suspension (0.1 ml) of pectin was added to each well in nutrient agar, and the cultures were incubated at 37°C for 3 days. The clear zones were then measured (Daoud *et al*., 2013).

Statistic analysis of data

All observations were presented as Mean ± SE (Standard error). Sigma Plot© (Version12.0) was run to analyze data. $P < 0.05$ was considered as significant. Matching three samples t-test was performed to check whether there were any significant differences.

3.2.6. Molecular identification of unknown laboratory bacterial strains

Generally, universal bacterial identification methods can be achieved using the 16S ribosomal RNA(r RNA) gene. This technique has been used for many years in bacterial taxonomy to measuring the similarity between bacterial isolates.

3.2.6.1. Extraction of bacterial DNA

1- A nutrient broth medium were prepared and incubated overnight at 37°C. After the incubation period, 1ml of media was transferred in a sterile Eppendorf tube and centrifuged at 6000 x g for 2 min at room temperature and the supernatant was discarded completely.

2- The pellet was resuspended in 100µl of buffer R1 and mixed completely by pipetting up and down. 20 µl of lysosyme was added and mixed well and incubated at 37°C for 20 min.

3- The mixture was centrifuged at 10,000xg for 3 min and the supernatant was completely discarded.

4- 180 µl of buffer R2 was added to the pellet and then 20 µl of proteinase K was added and incubated at 65°C for 20 min in a water bath. 400 µl of buffer BG was added and mixed well and then incubated for 10 min at 65°C. After the incubation period 200 µl of absolute ethanol was added and mixed.

5- The sample was transferred into a column tubes and centrifuged at 10,000xg for 1 min whilst the filtrate was discarded. The column was then washed by the addition of 750 µl of wash buffer and centrifuged at 10,000xg for 1 min whilst the passing was discarded.

6- Finally, the column was placed in a clean microcentrifuge tube; 70µl of elution buffer was added and centrifuged at 10,000xg for 2 min to elute DNA. DNA was stored at -20 °C.

3.2.6.2. Agarose gel electrophoresis

DNA fragments were separated by using 1 % agarose gel. These gels were prepared in the by dissolving 0.5g of molecular biology grade agarose in 50 ml of 10x TAE buffer. The mixture was heated in a microwave approximately 3 minutes until dissolving the agrarose and the solution was cooled. Then, 2.5µl of ethidium bromide was added to the mixture before setting the solution in gel tray, and then the gel was poured in the gel rack. After that, the comb was kept at room temperature. The gel was flooded in 10x TAE buffer. Samples were mixed with 2µl loading dye then were added to the wells. 6µl of Hyper Ladder was used in one well in order to find out the size of fragments. The samples were then undergone electrophoresis for 40 min operated at 80V. Digital images were taken using UVitec linked to a digital camera.

3.2.6.3. 16S rRNA gene

After isolation of genomic DNA from unknown bacteria, 16S rRNA genes were performed using polymerase chain reaction (PCR). Bacterial universal Oligonucleotide primers (Forward and Reverse) were used. The 16S rRNA gene was amplified with the universal fungal forward primer 16SUN1 FOR CCGAATTCGTCGACAACAGAGGATCCTGGCTCAG (34) and 16SUN1 REV CCCGGGATCCAAGCTTACGGCTACCTTGTTACGACTT (37) (Weisburg *et al*., 1991). Typically, the same PCR mixture in fungi was used in bacteria. The thermal cycling order which was used for the amplification of 16S rRNA gene in this experiment was as follows: initial denature at 94°C for 3 min to separate double stranded DNA into two single strands, followed by 30 cycles of DNA denaturation at 94°C for 1 min, and primer annealing at 60°C for 1 min. Afterwards, the temperature was decreased to allow primers to anneal. Strand elongation was conducted at 72°C for 5 min to allow the Taq polymerase to replicate the remaining single strand of DNA. Final elongation was done at 75°C for 5 min. The PCR reaction was achieved in electrophoresis on a 1 % agarose gels after finishing the

amplification processes of 16S rRNA gene. 10 µl of PCR product was mixed with 2 µl of Blue/Orange 6× loading dye and run on a 1 % agarose gel as described above. Besides, 6 µl of 1 Kb Hyper ladder loading in the gel to confirm the correct sized product.

3.2.6.4. Phylogenetic analysis

The samples were immediately sent to the University Medical School Core Genetics Unit for sequencing using 16S FOR /16S REV as sequencing primers. 16S rRNA gene sequences were compared in the Basic Local Alignment Search Tool (BLAST). All sequences were corrected by the Finch TV software to identify matches with existing characterized sequences.

3.3. Results and Discussion

3.3.1. Qualitative assay of pectinolytic bacteria

Seven bacterial species were found to grow on pectin as the only carbon source (see Table 3.1). Initial results showed that most bacterial species produce pectinase and break down apple pectin in PSAM. Most isolates were classified as good producers of pectinase by virtue of producing hydrolysis zones around colonies. The different species exhibited different growth rates, *Pseudomonas aeruginosa* showed clear zones at pH 7 only. This results in agreement with the results obtained by Latif and Sohail (2012) who reported that species of *Pseudomonas* can produce pectinase in pectin-rich medium. However, the same strain showed no clearing zones at pH 5. This may be due to the fact that *Pseudomonas aeruginosa* produced alkaline pectinases only (pH 7).

Table 3.1: Enzyme production by bacteria in PSAM after 48 hours incubation.

Bacteria	Pectate lyase production (pH 7.0)	Polygalacturonase production(pH5.0)
Pseudomonas aeruginosa	positive	Negative
Bacillus cereus (B1)	positive	positive
Staphylococcus aureus	Negative	Negative
Bacillus thuringiensis (B2)	positive	positive
Escherichia coli (E.coli)	positive	positive
Cupriavidus necator	positive	positive
Bacillus megaterium	positive	positive
Rhizobium sp.	positive	positive

Our results are confirmed by the studies of Kumar *et al*.,(2005) which indicated that *Pseudomonas aeruginosa* does not produce pectinase. *Bacillus cereus* was found to produce two forms of pectinase. Positive pectinase production from *Bacillus cereus* has also been confirmed by Venkata Naga raju and Divakar (2013). In the case of *Staphylococcus aureus* no clear zones was observed and lower growth was detected. These results disagree with those of Naga raju and Divakar (2013) who found that coccal species, including *Staph. aureus* are active pectinase producers. *B.megaterium* appeared to be a strong pectinase producer. Figure 3.1 shows clearly two representative plates of *B.megaterium* grown on pH 7.0 and pH5.0.The present finding support Bertagnolli *et al*.,(1996) study which concluded that culture filtrate from *B.megaterium* contained pectinase activity.

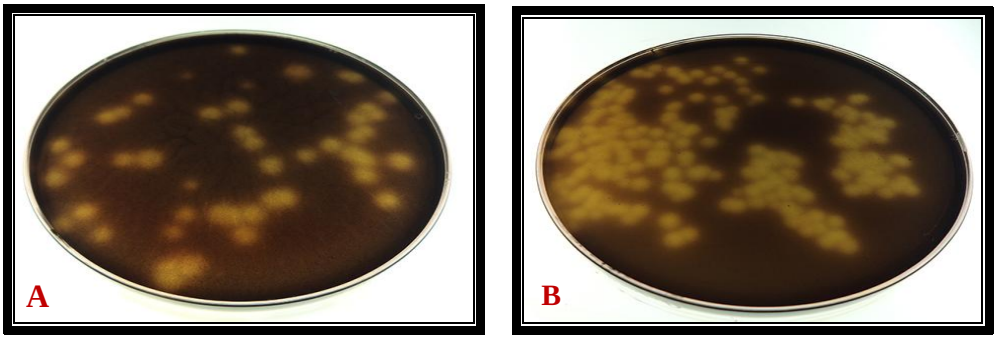


Figure 3.1: *Bacillus megaterium* in PSAM medium (A) pectin medium at pH 7.0 (B) pectin medium at pH 5.0 after 48 hours.

3.3.2. Qualitative assay of pectinolytic fungi

Most fungal cultures were shown to be good pectinase producers. The fungal pectinase activities have been shown in Table 3.2. *Aspergillius flavus* , *Aspergillius oryza, Penicillium verrclusium* and *Pencillium daleae* were shown to produce pectinase in two forms. A study has been reported that the *Penicillium* and *Aspergillus* species are able to produce pectinase (Favela-Torres *et al*., 2006). Several studies have been demonstrated that among many microorganisms, *Aspergillus* species are widely used to produce huge amount of pectinase (Teixeira *et al*., 2000; Martínez-Trujillo *et al*., 2009). Furthermore *Aspergillius niger* is considered the main fungus used for the industrial production of pectinolytic enzymes (Latif and Sohail, 2012).

Table 3.2: Enzyme production fungi in PSAM after 2 days of incubation.

Fungi	**Pectate lyase production (pH 7.0)**	**Polygalacturonase production(pH5.0)**
Trichophyton mentagrophytes	Negative	Negative
Alternaria tenuissima	positive	positive
Aspergillius flavus	positive	positive
Penicillium verrclusium	positive	positive
Motriella amobia	positive	positive
Aspergillius oryza	positive	positive
Penicillium daleae	positive	positive
Aspergillius niger	positive	Negative
Mucor hiemils	positive	positive
Acromonium strectium	positive	positive

The results presented in this Thesis show that *Aspergillius niger* grew poorly and was not able to produce pectinase at pH 5. This trend was not expected and it could be the pH 5 was not a favourable pH for pectinase production in *Aspergillius niger.* Production of pectinase capable of degrading apple pectin was detected also with *Acromonium strectium, P.*

verrclusium, Alternaria tenuissima and *Motriella amobia* (Fig.3.2). The opposite results was observed using *Trichophyton mentagrophytes* which was unable to produce the two forms of pectinase, likely because its nutritional needs were little, and it might be provided by another substrate in the medium.

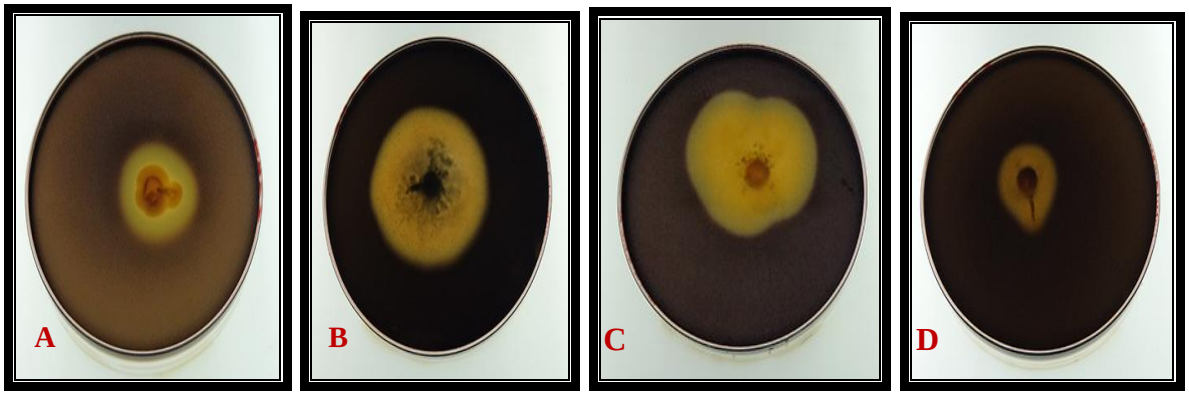


Figure 3.2: Fungal pectinase production in PSAM medium pH 5 after 2 days incubation (A) *Acromonium strectium,* (B) *Alternaria tenuissima* (C) *Motriella amobia* and (D) *Penicillium verrclusium.*

3.3.3. Quantitative assay for pectinase derived from pectinolytic bacteria

Pectinase production and growth by *B.megaterium* was evaluated on two types of broth medium in order to determine which medium supports pectinase production.

3.3.3.1. LB

For the pectinase production in broth medium *B.megaterium* was selected as the most active isolate. Initially, the strain was evaluated on LB medium supplemented with apple pectin as the carbon source. As it can be seen in Figure 3.3, there was a gradual rise in enzyme production during 60 hours. In addition, pectinase production reached a maximum value after 60 hours. Whereas, the pectinase level in the control remained low and stable.

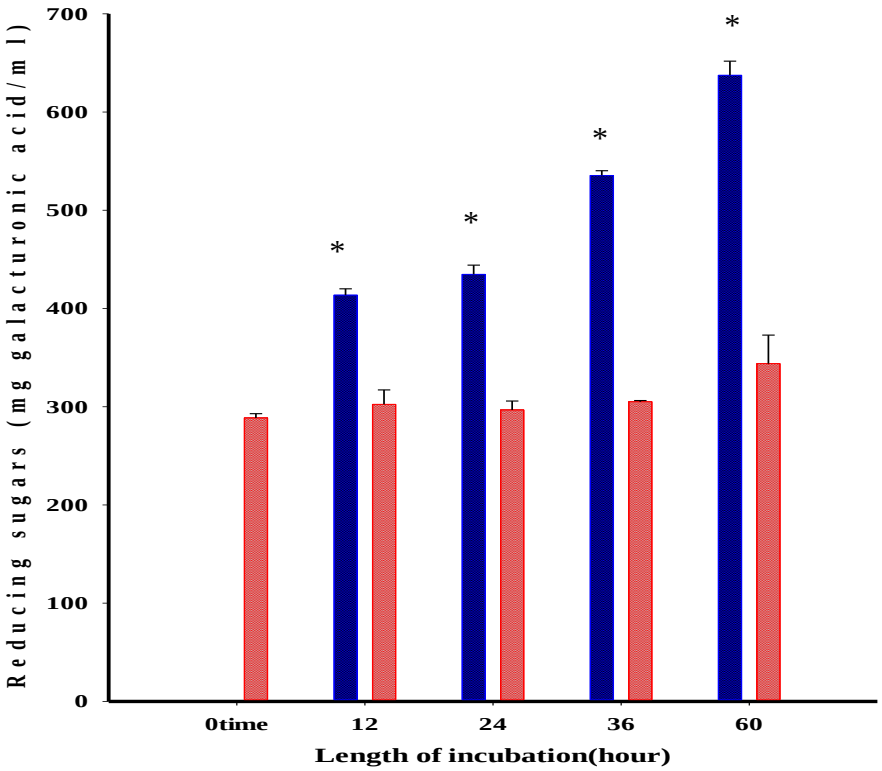


Figure 3.3: pectinase activity produced by *B.megaterium* in LB medium supported by apple pectin. *B.megaterium* ( ) and control( ). Means of triplicates (±) standard error.

*significant difference from the control ($P < 0.05$).

It can be concluded that *B.megaterium* grew well on LB medium plus pectin and produced different levels of pectinase. Moreover, the pectinase was secreted in early stages (12 hours.) , a fact which allows quick detection of degraded products. As indicated by Kashyap *et al*.,(2000); LB medium with 0.25% pectin is considered the best medium for pectinase production. A significant difference ($P<0.05$) in pectin degradation was observed throughout the incubation period. Figure 3.4 shows that culture pH was substantially increased through 60 hours to reach the highest value 9.6; this trend corrolated with the pectinase production trend.This fact suggests that pectinase activity is directly infuenced by the pH of medium. Significant difference ($P<0.05$) in pectin degradation was observed in tested incubation period except after 60 hours.

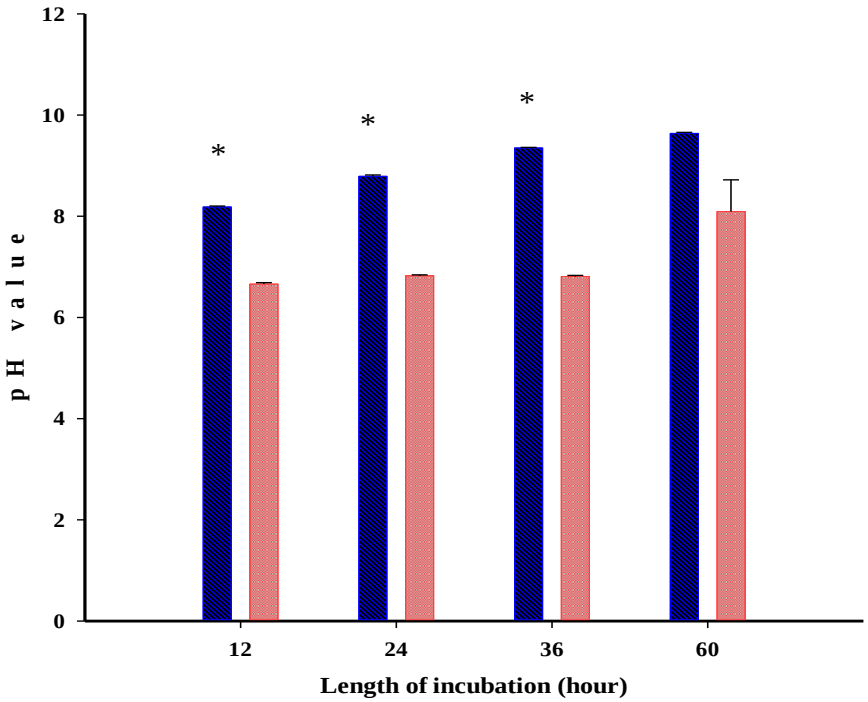


Figure 3.4: The pH values obtained when *B.megaterium* was grown in LB medium containing apple pectin. *B.megaterium*( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

3.3.3.2. YEP

The strain was also evaluated on YEP medium supplemented with apple pectin as the carbon source. Figure 3.5 refers to the pectinase production in YEP medium through 60 hours. There was a considerable increase in pectinase production during 60 hours. A maximum value recorded at 24 hours incubation. Furthermore, there was marked pectinase production in the early stages of growth (at 12 and 24hours), followed by a gradual decrease at 36 and 60 hours. However, the control remained low and stable.

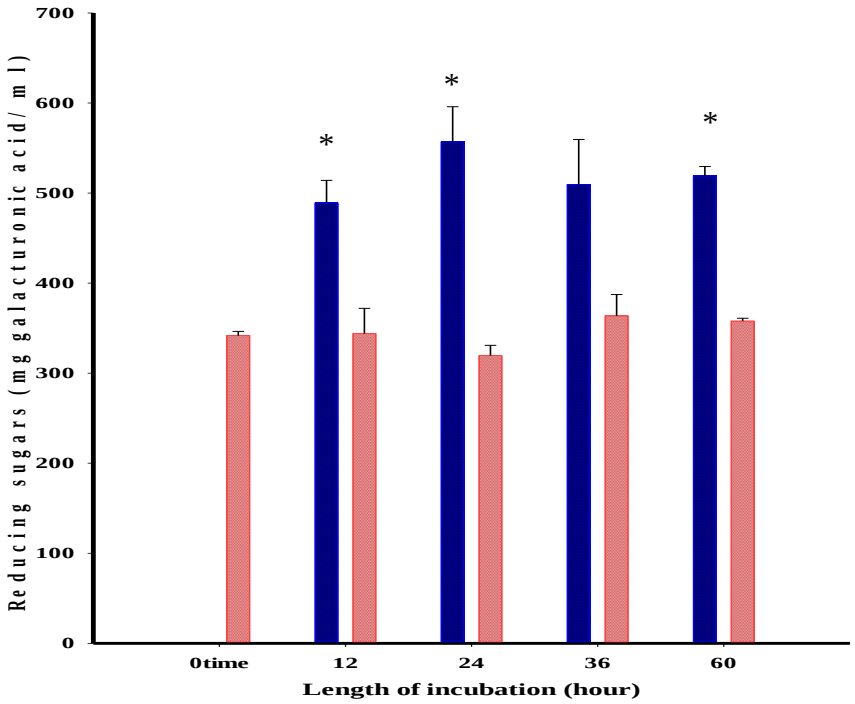


Figure 3.5: Pectinase activity produced by *B.megaterium* in YEP medium supported with apple pectin. *B.megaterium*( ) and control ( ).Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

YEP medium with 0.25% pectin as the greatest medium for pectinase production has been reported by Kashyap *et al*., (2000). A Significant difference ($P<0.05$) in pectin degradation was observed in tested incubation period except after 36 hours.The results given in Figure 3.6 show the value of pH in YEP medium.

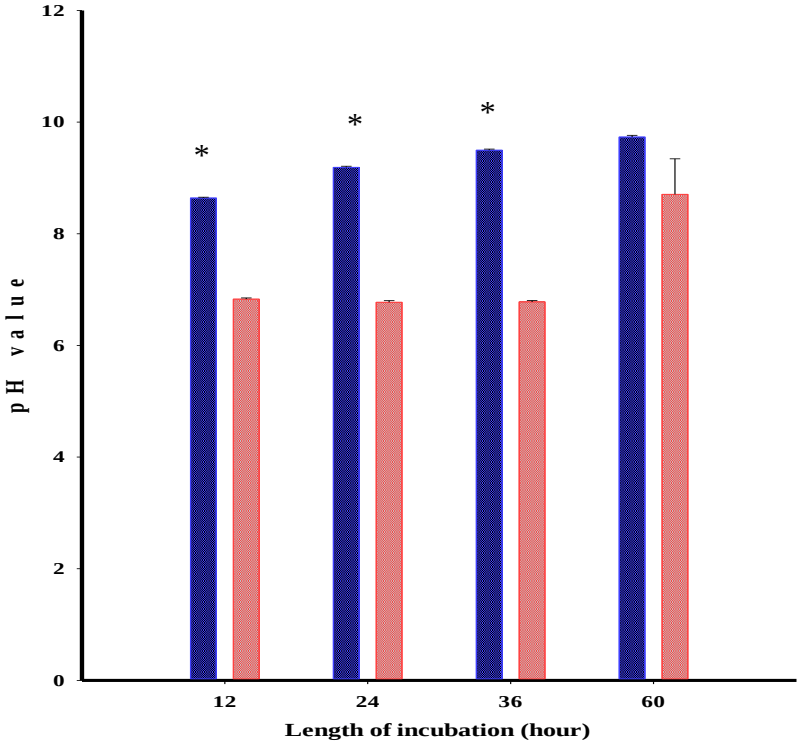


Figure 3.6: pH values obtained by *B.megaterium* in YEP medium supported with apple pectin. *B.megaterium* ( ) and control ( ). Means of triplicates (±) standard error.

*significant difference from the control ($P < 0.05$).

The levels of alkalinity found in inculcated culture higher than those found in control. A maximum value was observed after 60 hours and it was 9.7. Based on these results, the alkalinization of YEP medium correlated with ammonia production. The results presented here suggest that the production of pectinase is influenced by the conditions of medium such as pH (Silva *et al*., 2005).

3.3.3.3. Effect of salts on pectinase production in LB and YEP medium

The influence of two salts on pectinase production has been studied. The results provided in Figure 3.7 and 3.8 show that the addition of $CaCl_2$ and Mg_2SO_4. $7H_2O$ to LB medium produced an increase in pectinase production compare to the control.

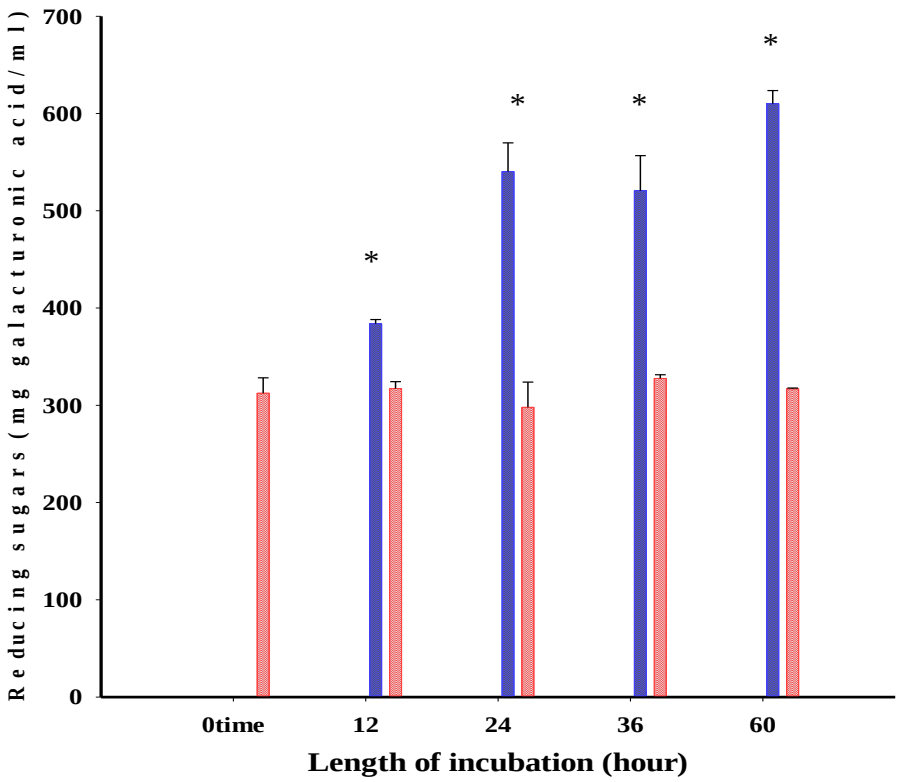


Figure 3.7: Effect of $CaCl_2$ on pectinase production by *B.megaterium* in LB medium supported with apple pectin. *B.megaterium* in LBPC (▬) and control in LBPC (▬). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

It can be clearly seen that $CaCl_2$ and Mg_2SO_4 enhanced pectinase activity by *B.megaterium* in LB medium and supported enzyme production reached the highest value after 60 and 36 hours respectively.

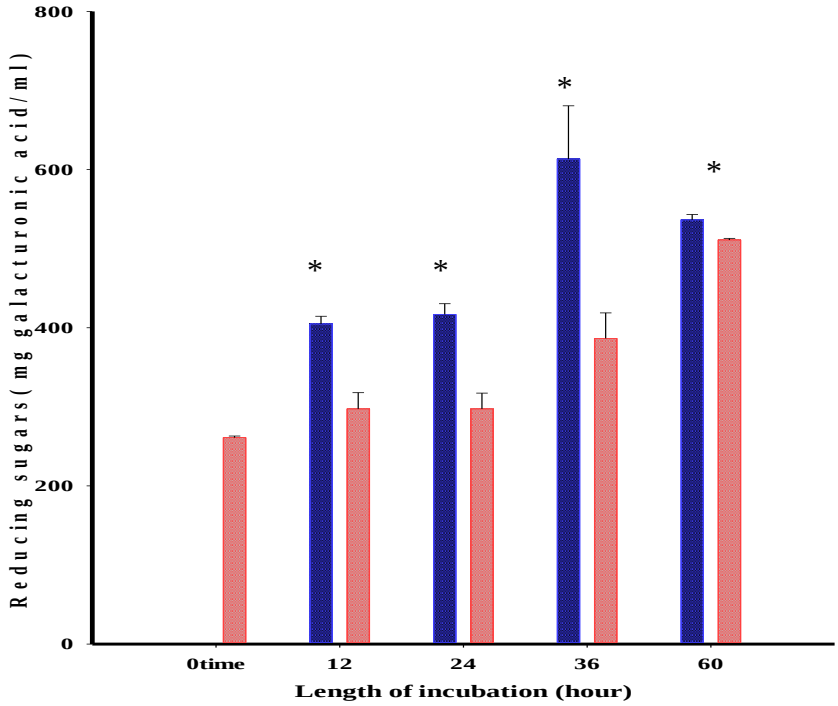


Figure 3.8: Effect of Mg_2SO_4 on pectinase production by *B.megaterium* in LB medium supported with apple pectin. *B.megaterium* in LBPM ( ) and control in LBPM ( ). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

In the case of the addition of $CaCl_2$, the culture achieved a gradual increase of pectinase production in early stages (at 12 and 24hours) then the level of enzyme decreased following by achieved maximum value after 60 hours of incubation. Whereas the addition of Mg_2SO_4. $7H_2O$ promoted pectinase production to rise gradually to reach the highest value after 36 hours of incubation, after which it decreased. Activity was observed over the incubation period in LB+ $CaCl_2$ and LB+ $Mg_2SO_4.7H_2O$; the salts improving pectinase production. A similar trend was observed in YEP medium (Fig. 3.9 and 3.10). The addition of $CaCl_2$ and Mg_2SO_4. $7H_2O$ to YEP medium resulted in a considerable increase in pectinase after 60 and 24 hours respectively.

Regarding the addition of $CaCl_2$, the levels of enzyme found in inoculated culture was higher than those found in control. This fact suggests that YEP with $CaCl_2$ was favorable medium for the growth of *B.megaterium*. In addition, This explains why YEPC medium is regarded as a useful additive for pectinase production.

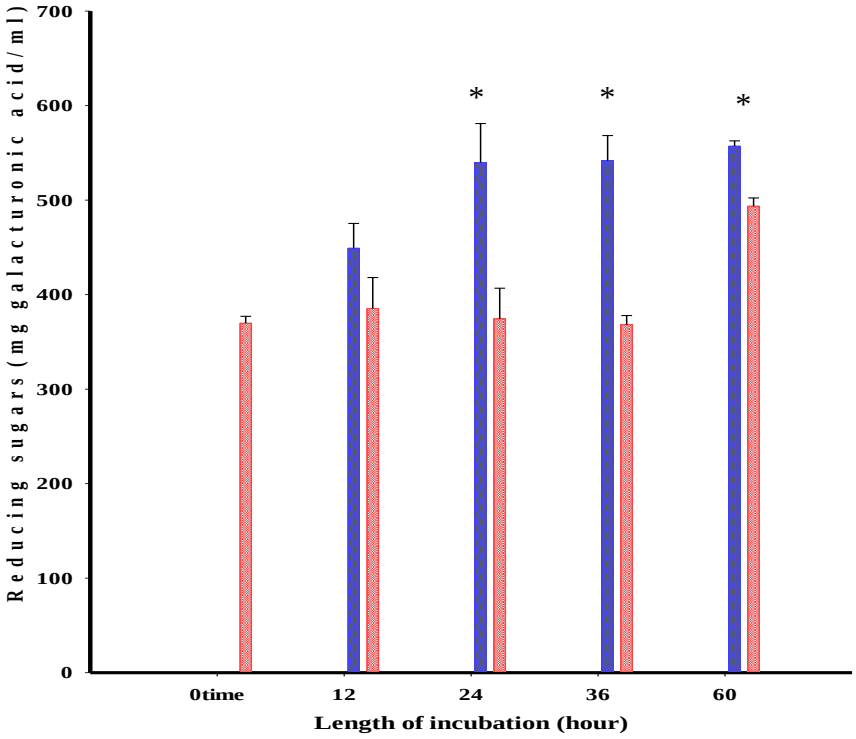


Figure 3.9: Effect of $CaCl_2$ on pectinase production by *B.megaterium* in YEP medium supported with apple pectin. *B.megaterium* in YEPC ( ) and control in YEPC ( ). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

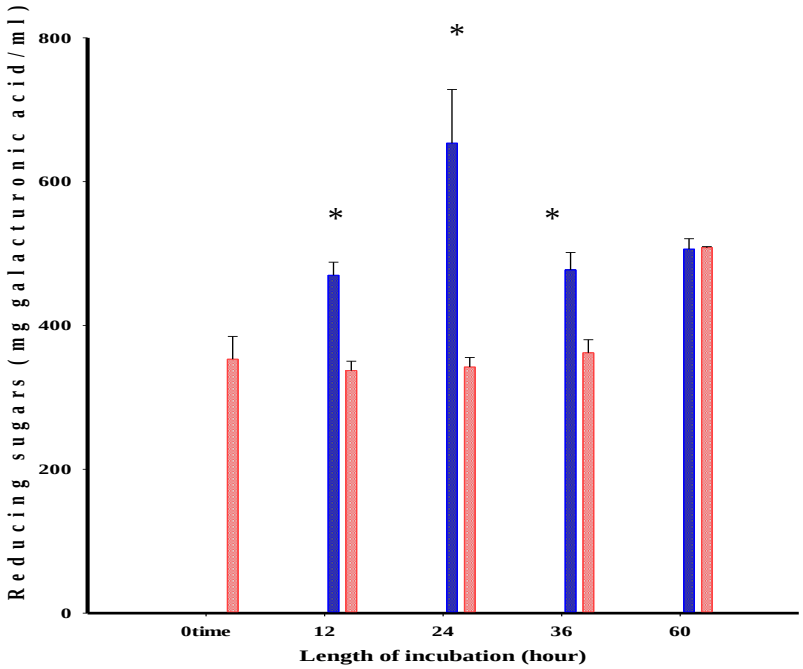


Figure 3.10: Effect of Mg_2SO_4 on pectinase production by *B.megaterium* in YEP medium with apple pectin. *B.megaterium* in YEPM ( ) and control in YEPM ( ). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

Similarly, pectinase production occurred throughout the incubation period uisng YEPM medium and was expected (Fig. 3.10). A notable rise in reducing sugars occurred after 12 hours of incubation, followed by a decrease at 36 and 60 hours. In the same time the control value remained stable, except after 60 hours. It can be concluded that addition of salts to LB and YEP medium resulted a significant levels of pectinase and the supplementation of the salts promoted pectinase production. This results in agreement with the work of Kashyap *et al*., (2000).

A change in the pH of LB medium when amended with salts was observed (Fig. 3.11). The pH of the culture medium increased over the incubation period. This Figure shows a gradual increase in pH value to reach maximum value after 60 hours of incubation following addition of the salts. In the case of both salst, the maximum pH value was achieved after 60 hours at 9.5. It can be clearly seen that the alkalinity of medium reached the highest value in both salts after 60h of incubation in LB medium.

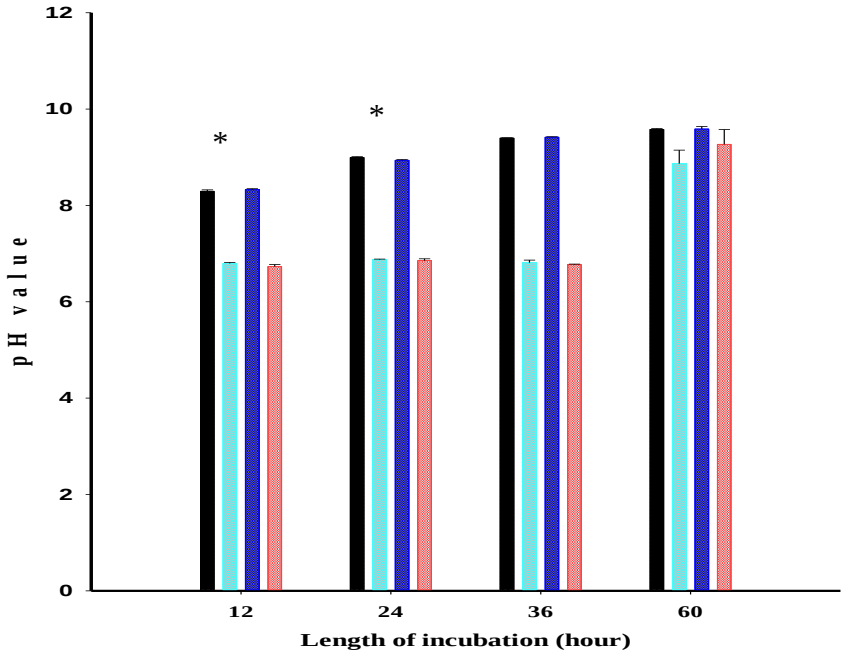


Figure 3.11: pH values obtained by *B.megaterium* in LB medium supported with $CaCl_2$ and Mg_2SO_4 . *B.megaterium* in LBPC ( ) and control in LBPC ( ). *B.megaterium* in LBPM ( ) and control LBPM ( ). Means of triplicates (±) standard error. *significant difference from LBPC and LBPM ($P < 0.05$).

Change in pH value in YEP medium amended with salts was also determined. In Figure 3.12 the value of pH increased gradually and the highest value was found after 60 hours. The maximum was 9.7 in YEP +$CaCl_2$ and 9.5 in YEP + Mg_2SO_4. A notable fact arising from this work is that an increase in pH value was observed in all cases in LB and YEP media and the changes in pH values were produced in the early stages of pectin degradation.

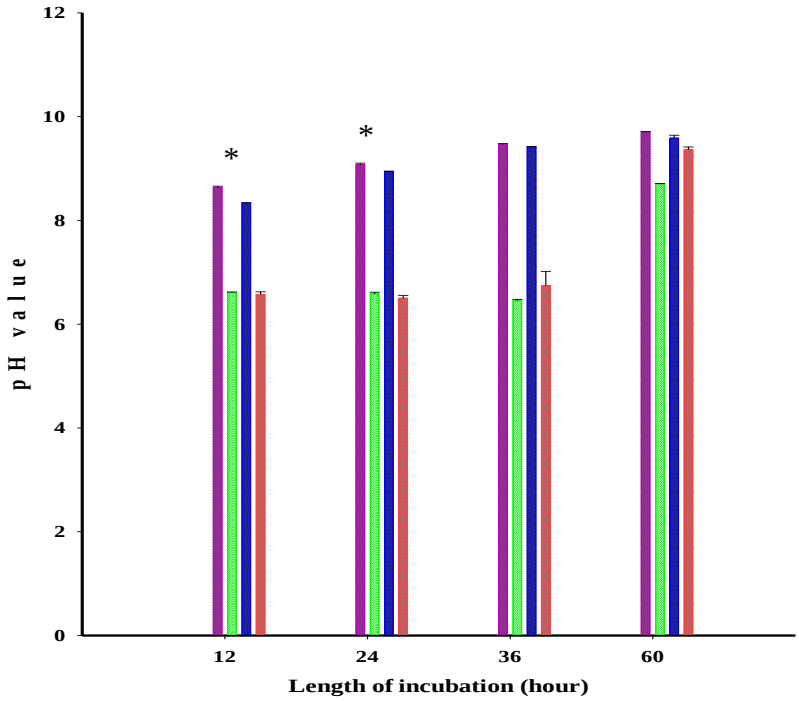


Figure 3.12: pH values obtained by *B.megaterium* in YEP medium supported with $CaCl_2$ and Mg_2SO_4 . *B.megaterium* in YEPC ( ) and control in YEPC ( ). *B.megaterium* in YEPM ( ) and control in YEPM ( ). Means of triplicates (±) standard error.

*significant difference from YEPC and the YEPM ($P < 0.05$).

The relationship between pectinase production and the change in pH was studied in all types of test media. Table 3.3 shows the association of pectin degradation and pH values.

There was a slight difference between the point of maximum pectinase production and the point of highest pH value. This fact explains that pectinase production could be modulated by pH of medium particularly in the case of LB, LBPC and YEPC media. However, in YEP, LBPM and YEPM enzyme production was seen to be unrelated to the pH value. When apple pectin was used as the carbon source, growth of *B.megaterium* was increased, indicating that *B.megaterium* was able to degrade the pectin enough to support its growth (Fig. 3.13).

Table 3.3: The relationship between pectinase production and pH.

Type of medium	The time of reach maximum value of pectinase production	The time of reach maximum value of pH
LB	At 60 h.	At 60 h. (9.6)
YEP	At 24 h.	At 60 h. (9.7)
LBPC	At 60 h.	At 60 h. (9.5)
YEPC	At 60h.	At 60h. (9.7)
LBPM	At 36 h.	At 60h. (9.5)
YEPM	At 24h.	At 60 h. (9.5)

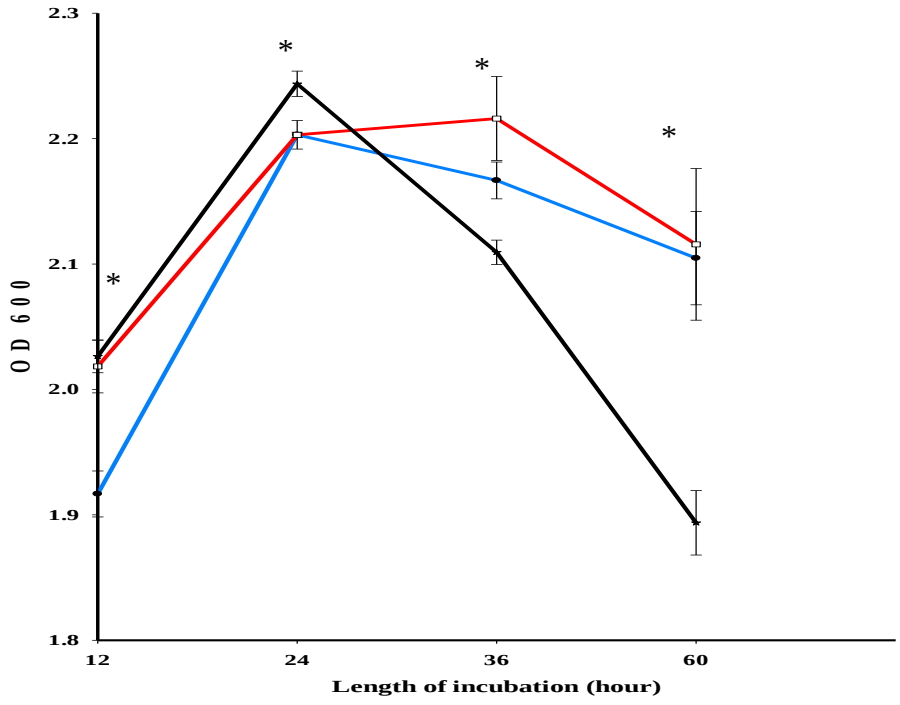


Figure 3.13: Growth curves of *B.megaterium* grown in LB medium supplemented with $CaCl_2$ and Mg_2SO_4 measured at optical density of 600 nm. *B.megaterium* in LB ( ) , *B.megaterium* in LBPC ( ) and *B.megaterium* in LBPM ( ). Means of triplicates (±) standard error. *significant difference from LB, LBPC and LBPM ($P < 0.05$).

Figure 3.13 shows that cell growth increased after 12 and 24 hours of incubation then the levels of growth decreased in LB and LBPM media. However, it remained stable in the case of LBPC. A similar result was obtained for YEP medium (Fig. 3.14). The results show that there was a significant growth after 12 hours of incubation after that this time, growth decreased gradually. However, there was no association seen between growth and the pectinase production by *B.megaterium* in all cases of LB and YEP media.

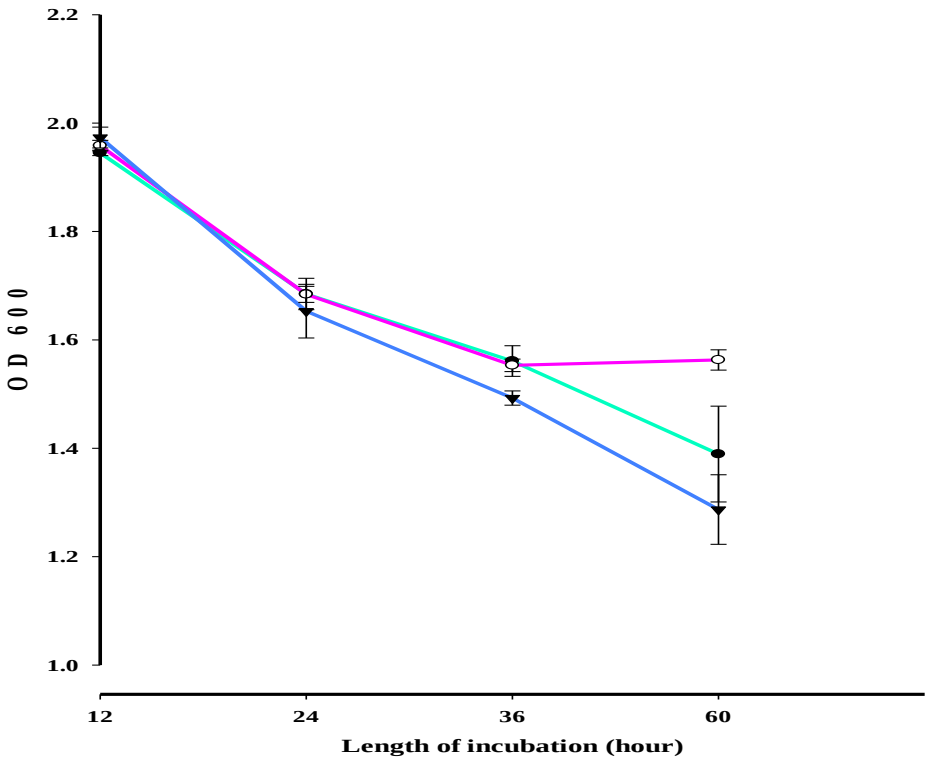


Figure 3.14: Growth curves for *B.megaterium* grown in YEP medium measured at optical density of 600 nm. *B.megaterium* in YEP( ) , *B.megaterium* in YEPC ( ) and *B.megaterium* in YEPM ( ). Means of triplicates (±) standard error.

3.3.4. Quantitative assay for pectinase derived from pectinolytic fungi

Figure 3.15 shows pectin degradation by *A.oryza.* Initially, a marked increase in pectinase was seen, with the amount peaking after 2 days of incubation, which was reduced after 3 and 5 days, whereas the control remained stable. In addition, *A.oryza* successfully grew in apple pectin to produce the enzyme. This result is in agreement with Benoit *et al*., (2012) who pointed out that *A.oryza* grows on pectin and it has pectinolytic potential and industrial use for pectinase production.

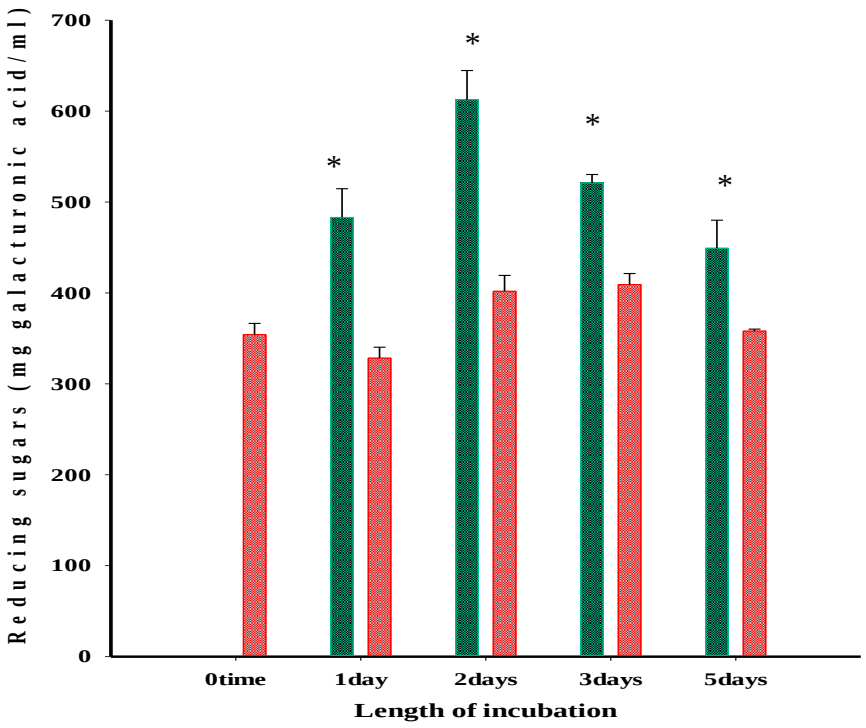


Figure 3.15: Pectinase activity produced by *A.oryza* in L-asparagine medium supported with apple pectin. *A.oryza* ( ) and control( ). Means of triplicates (±) standard error.

*significant difference from the control ($P < 0.05$).

3.3.4.1. Evaluation of pH

Figure 3.16 shows that pH was increased and peaked 8.3 after 3 days of incubation.

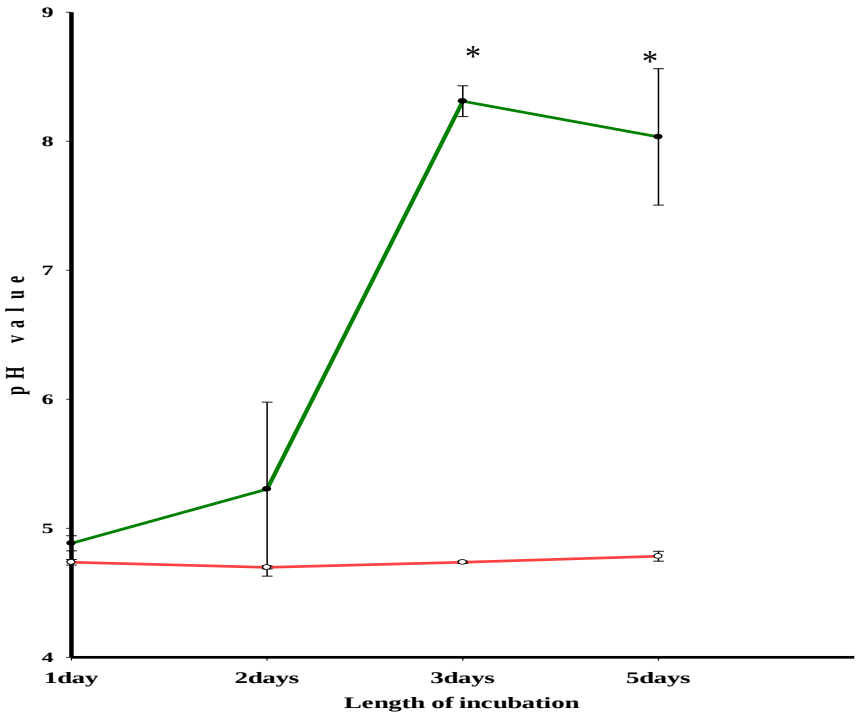


Figure 3.16: pH values obtained by *A.oryza* in L-asparagine medium supported with apple pectin. *A.oryza* (—●—) and control (—○—). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

This value decreased after 5days. However, the control was stable. It can be seen that pectinase activity did not reached the highest value at the point of maximum pH value (after 3 days).

3.3.4.2. Evaluation of growth

The growth was evaluated by the mycelium density combined with measurement of the biomass. The results provided in Figure 3.17 illustrate the optimal growth of *A.oryza* on pectin medium.The results show that there was a significant growth of the strain and the fungus grew very well to peaked the highest value after 5days. On the other hand, *A.oryza* show a decrease in biomass after 3 days. Generally, the pectin medium promoted *A.oryza* growth.

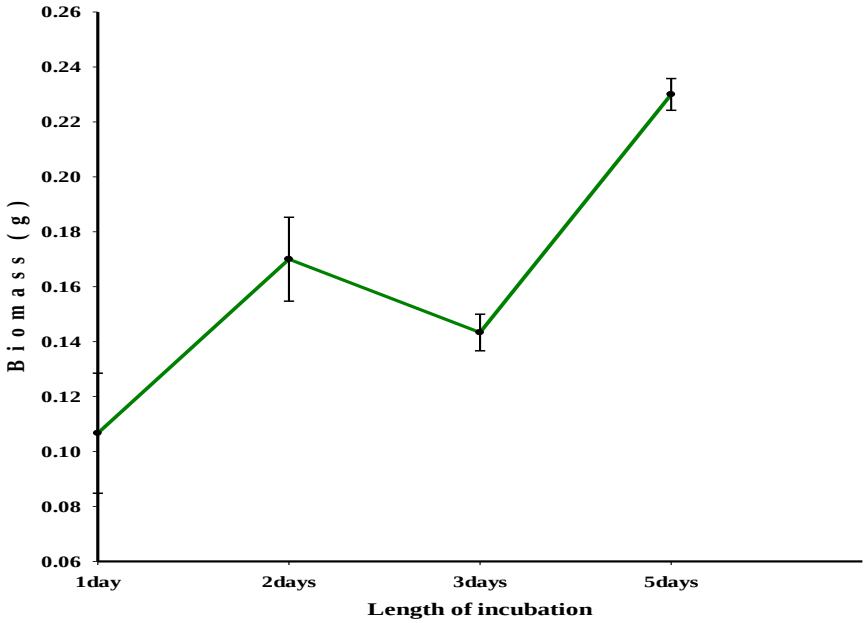


Figure 3.17: Biomass produced by *A.oryza* in L-asparagine medium supported with apple pectin. Means of triplicates (±) standard error.

3.3.5. Chemical analysis

Samples from pectin were analyzed to determine macronutrients decomposition products from pectin (Nitrate and ammonium).

3.3.5.1. Determination of nitrate

Figure 3.18 shows the nitrate levels in agricultural soil amended with pectin and control. The nitrate production from the oxidation of ammonium reached maximum value in zero time then the value was increased dramatically. Surprisingly, the levels of nitrate in control were higher than the treatment in all weeks of incubation. In addition, the highest nitrification peaked in control at 4 weeks.

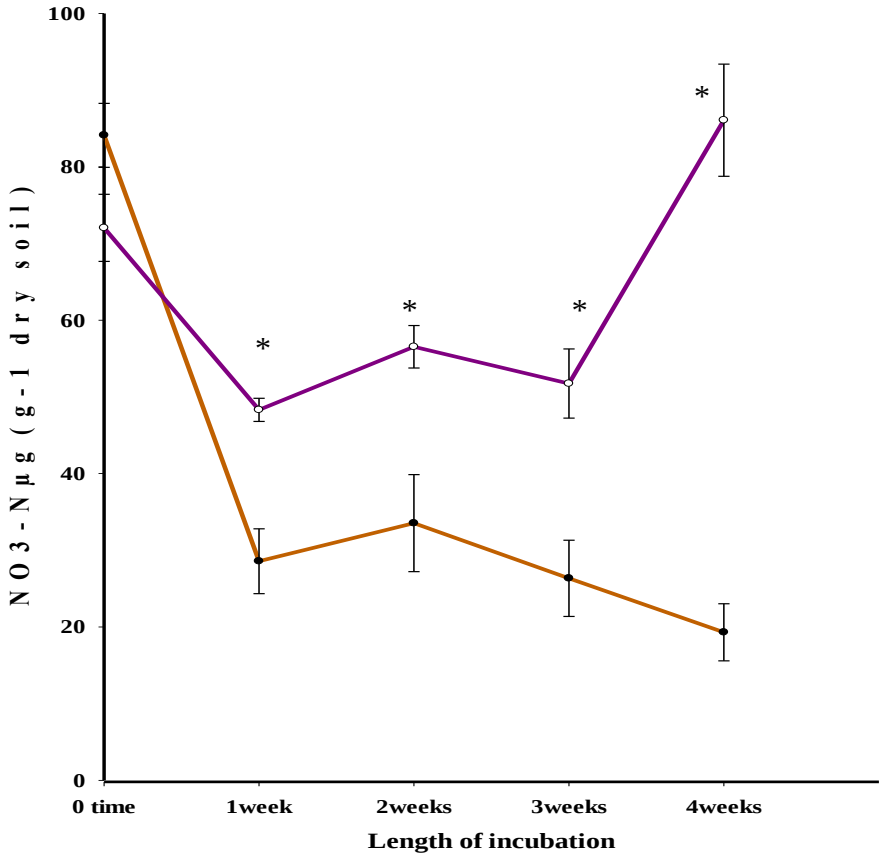


Figure 3.18: Nitrate production from the hydrolysis of pectin. Treatment soil (—●—) and control(—○—). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

3.3.5.2. Determination of ammonium

Figure 3.19 shows the amount of ammonium produced from agricultural soil amended with pectin. The amount of ammonium in the treatment was higher than control in week two and week three. However, the levels of ammonium peaked, the highest value in the treated sample occurring after 2weeks. There is no significant difference between treatment and control.

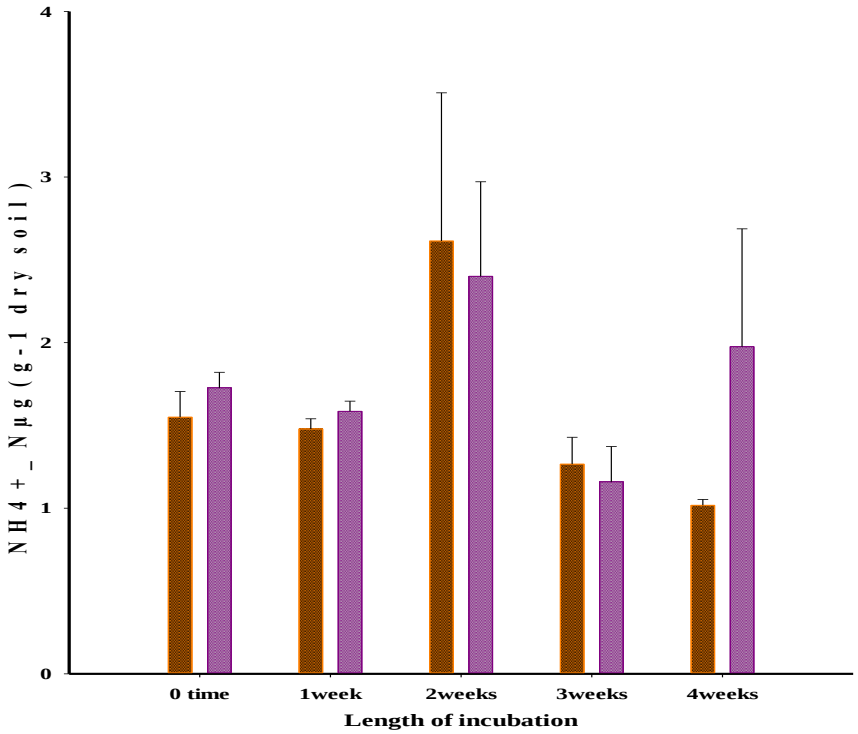


Figure 3. 19: Ammonium production from the hydrolysis of pectin. Treatment ( ) and control ( ). Means of triplicates (±) standard error.

3.3.6. The antimicrobial activity of pectin

Table 3.4 shows antibacterial effects of pectin aginst some pathogens partically the bacteria cause infection in the wounds. The results show that the pectin had considerable antibacterial activity against *Escherichia coli*, *Staph.aureus* and *Staph.aureus* MRSA3 which act as the most flora cause wound infection. In addition, they are common human pathogens.

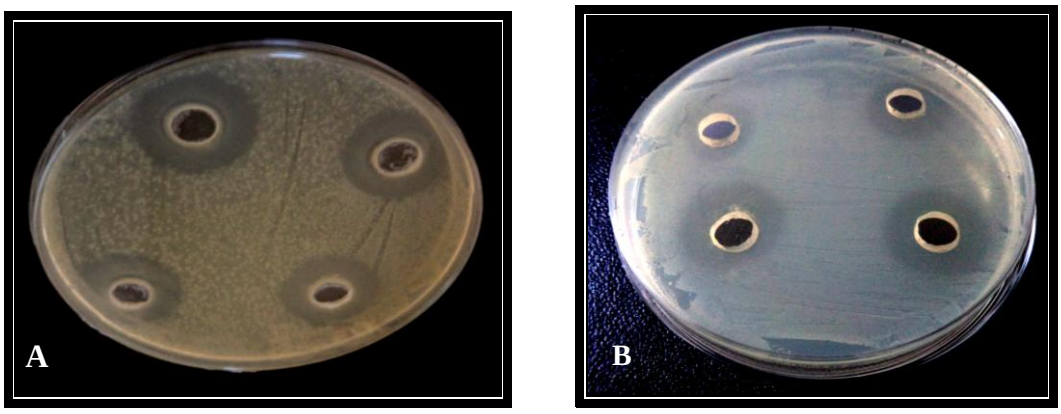


Figure 3.20: Antibacterial activity of pectin against some bacterial flora in the wounds (A) *Staph.aureus* and (B) *Staph.aureus* MRSA3 after 48hours of incubation.

The more surprising is pectin can be used against resistant bacteria such as *Staph.aureus* MRSA3 which is considered a resistant bacterium to common antibiotics (Fig. 3.20). However, pectin had no effect against *Pseudomonas aeruginosa.* This fact considered the pectin has narrow range of antibacterial activity and affected on specific wound flora of bacteria.

Table 3.4 : Antibacterial activity of pectin.

Bacteria	**Antibacterial effects**
Escherichia coli	Positive
Staphylococcus aureus	Positive
Staphylococcus aureus MRSA3	Positive
Pseudomonas aeruginosa	Negative

The data from this study explain that there is an obvious antibacterial effect of pectin against bacteria related to wound infection (Fig. 3.21).

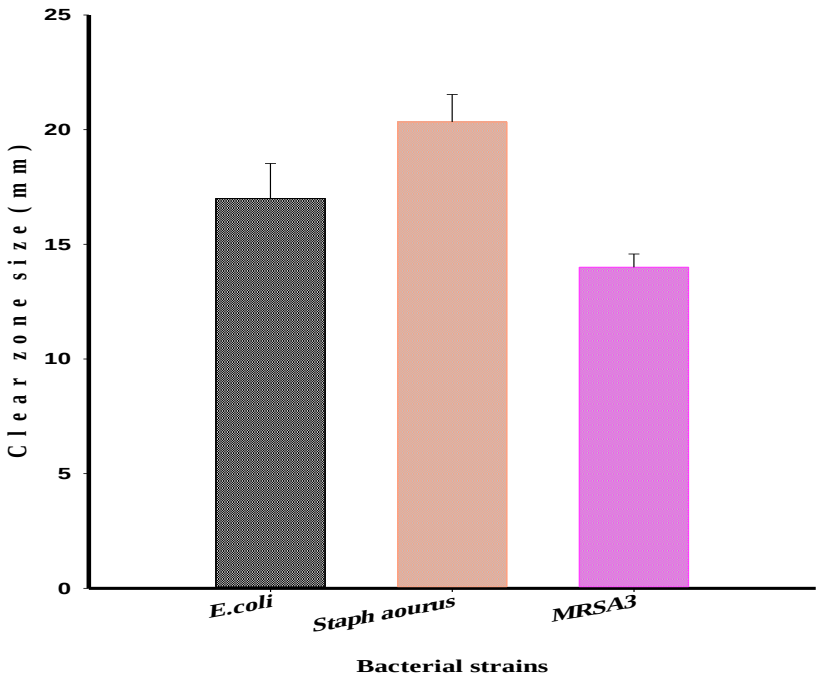


Figure 3.21: Clear zone size (mm) produced from bacterial strains after 48 hours of incubation.

This suggests that the pectin is natural substrate accessible in the different types. Moreover, the pectin probably natural antimicrobial can be used against these bacterial strains as safe treatment. Antibacterial effects of pectin against bacteria confirmed by Daoud *et al*., (2013). Far too little attention has been paid to use of pectin against pathogenic bacteria. It can be concluded that development of our knowledge about the pathogenicity and virulence of *E.coli*, *Staph.aureus* and *Staph.aureus* MRSA3 is needed. In addition, the resistance of bacteria against antibiotic, the costs of medicine, side effect of antibiotic and limited new discovery of antibiotics needs alternative therapy.

3.3.7. Phylogenetic identification of unknown laboratory bacterial strains

The unknown laboratory isolates used in pectinolytic activity were identified using molecular techniques. Two strains of unknown bacteria were identified using 16S FOR and 16S REV primers. They were designed as B1 and B2. Agarose 1 % gel was used to check the expected size of amplified product. Figure 3.22 shows the successful PCR products.

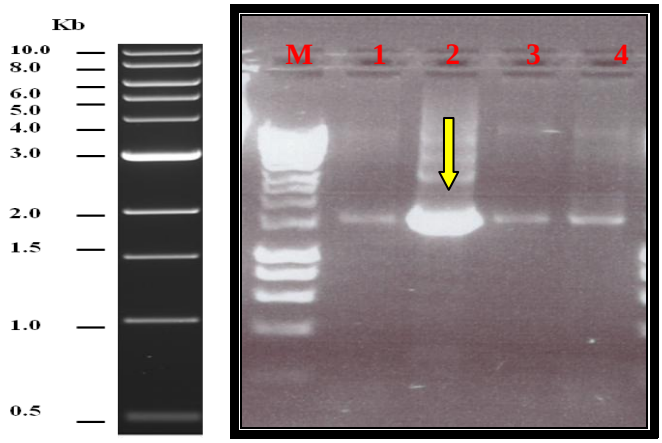


Figure 3.22: Product of 16S rRNA gene from PCR produced from *Bacillus cereus.* Arrow indicates bacterial product from the gel.

The isolates were identified as *Bacillus cereus* and *Bacillus thuringiensis* with similarity 99 % and 98% respectively (Table 3.5). Blast analysis of 16S rDNA sequence of B1 gave a similarity of 99% with *Bacillus cereus* (Fig.3.23).

Table 3.5:16rRNA sequence analysis of unknown bacteria.

Representative sequence	Closest matches identification	Sequence identity	Length of Sequence (bp)	NBCI (Accession number)
B1	*Bacillus cereus*	99%	1525	KF672365.1
B2	*Bacillus thuringiensis*	98%	789	EU588682.1

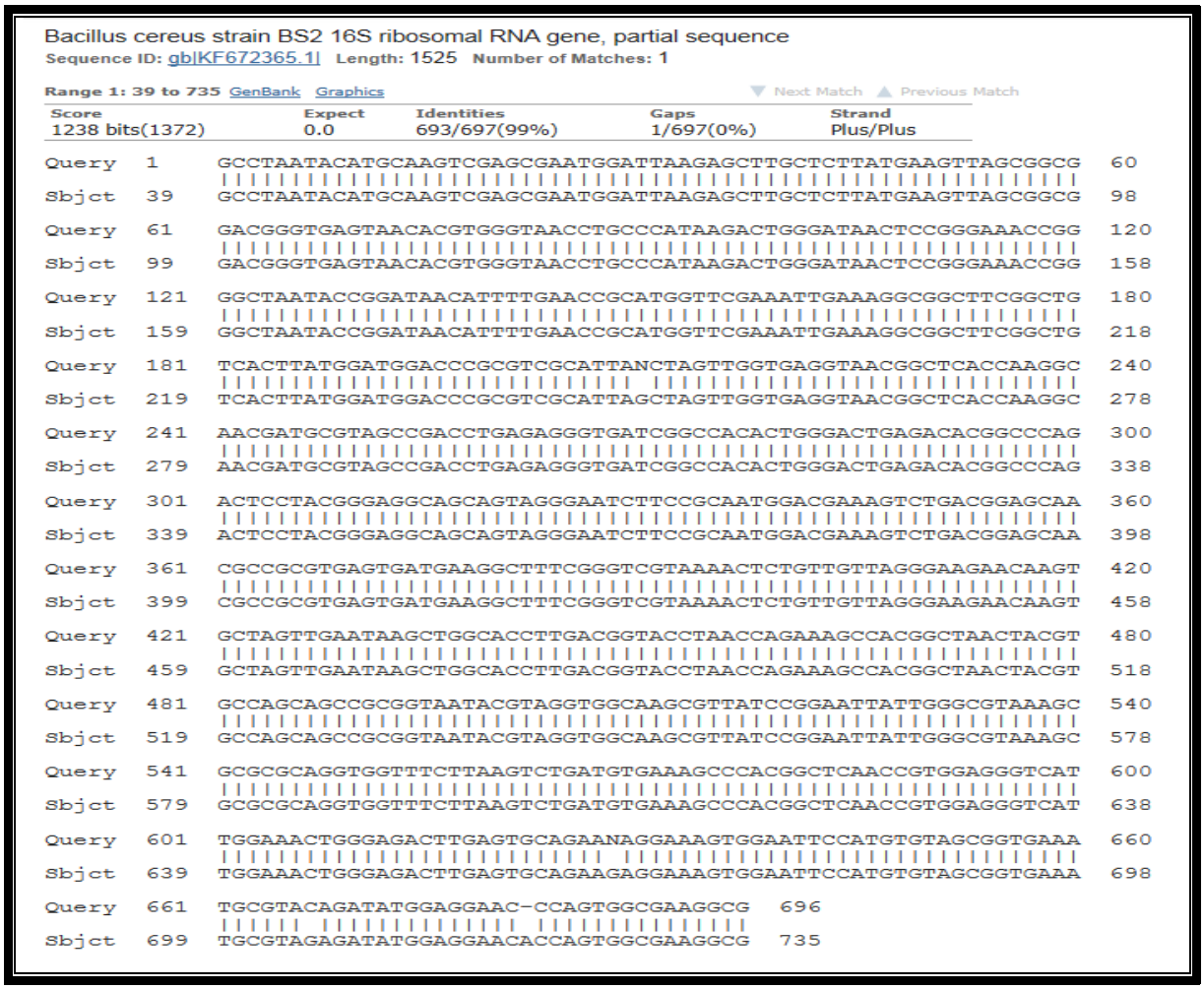


Figure 3.23: Sequence of *Bacillus cereus* (achieved after the amplification of whole cell genome) referring to the affiliations through BLAST studies .The "Query " referring to the line when input sequence the "Subject" refers to sequence of line matching.

CHAPTER 4

CHAPTER 4
STUDIES ON MICROBIAL DEGRADATION OF ALGINATE

4.1. Introduction

Many recent studies have confirmed that the alginates are generally extracted from seaweed and they are used as gelling agents in many applications such as food, paper, biomaterials and pharmaceutical industries (Cao *et al*., 2007). The majority of alginates (which are used commercially) are obtained from three genera of seaweed; *Macrocystis*, *Laminaria* and *Ascophyllum.* Also, alginates are produced by two heterotrophic bacterial families: *Pseudomonadaceae* and *Azobacteriaceae.* According to an investigation by Wong *et al*., (2000); Bacterial alginates differ to seaweed alginates due to O-acetyl groups located on 2 or 3 position of D-mannuronic acid.

4.1.1. Source of alginates

The alginate from brown seaweed (Phaeophyta) and from *Azootobacter vinelandii* include random sequences of uronic acid blocks, while the alginate from *pseudomonas aeruginosa* and other species of *pseudomonas* contains L-guluronate only and it might have different numbers of D-mannuronate (Sutherland, 1995). Alginates are known as unbranched polysaccharides consisting of (1,4)-linked β-D-mannuronic acid (M blocks) and α-L-guluronic acid (G blocks) linked with glycosidic bond, arranged in two forms which are homopolymeric (MM- or GG-blocks) or heteropolymeric random sequence (MG- or GM-blocks) (Cao *et al.*, 2007).

4.1.2. Alginate degradation mechanism

The alginates can be depolymerised by the action of alginate lyase using the mechanisms of β-elimination to generate oligosaccharides. Oligosaccharides contain 4, 5-unsaturated glycosyluronic acid (4-deoxy-L-erythro-hex-4-eno-pyranosyl uronic acid) as non-reducing ends (Shimokawa *et al.,* 1997). The mechanisms target glycosidic 1→4 O-linkage which located between the monomers. Various oligosaccharides with unsaturated uronic acid (4-deoxy-L-erythro-hex-4-enepyranosyluronate at the non-reducing ends) and unsaturated uronic acid monomers can be formed as a result of alginate lyase action. Furthermore, by endolytic and exolytic alginate lyases, alginate can be transformed into unsaturated monosaccharides using the process of saccharification (Kim *et al.*, 2011).

4.1.3. Microbial alginate lyases

Alginate lyases are known as alginate depolymerases or alginases and they play important role in alginate degradation. In spite of all alginases achieving basically the same degradation mechanism on alginate; individual characteristics can be detected for each enzyme via its glycolytic bond preference. There are some marine animal and bacterial species producing two or more alginate lyases although most organisms producing a single alginate lyase. Due to the complex structure of alginates; microbial degradation for this material is not simple. Basically, the discarding and use of seaweed wastes are essential for the protection of marine environment and recycling of organic matter. It has been suggested that isolation of alginolytic microorganisms and use them to alginate degradation is significant for the successful recycling of seaweed wastes (ElAhwany and Elborai, 2012).

Alginate lyases have been recognized in several microorganisms such as marine bacteria, marine fungi and marine algae. The microorganisms which can use alginates as carbon source produce two types of alginate lyases: exolytic and endolytic alginases. Unless they show co-operation relationship with another organisms in order to breakdown the complex polysaccharide to small subunits (Abdel-Hafez *et al*., 2008). Although alginate lyases are found in marine organisms, also they have been detected in soil bacteria such as *Bacillus spp.* (Sutherland, 1995). Alginase lyase from *Haliotis, Photobacterium sp*. and *Pseudomonas aeruginosa* are considered as poly M lyase while those from *Klebsiella aerogenes* and *Pseudomonas alginovora* are poly G lyase (Sawabe *et al.*, 1997).

The alginate lyase can be produced by several bacterial strains such as marine bacteria, Gram-negative bacteria and Gram-positive bacteria. The majority of marine bacteria such as *P.alginovora* able to use alginate as carbon and energy source. In spite most of marine bacteria produce one alginate lyase, *P.alginovora* produces two types of lyases: poly (M) and poly (G) lyases. There is one difference between marine bacterial lyase and soil bacterial lyase which is marine lyase differs in the size and the substrate specificity. Alginate lyases can be found in Gram-positive soil bacteria such as *B.circulans* and Gram-negative soil bacteria such as *A.chroococcum, A.vinelandii* and *P.syringae.* Interestingly, alginate lyase also has been found in certain bacteriophages which specific for *Pseudomonas spp.* and *Azotobacter*. These phages use alginate lyases to penetrate the bacterial acetylated exopolysaccharides (Wong *et al*., 2000). Marine fungi have been investigated regarding

alginate lyases production. In fact, there are few species of marine fungi able to degrade alginates. These species belong to three species: *Dendryphiella salina*, *Asteromyces cruciatus* and *Corollospora intermedia* (Schaumann and Weide, 1990).

The primary goal of this Chapter was to isolate fungi and bacteria from detritus and decaying fronds from marine habitats. A further aim was screening for alginate degrading enzyme production. Classical molecular techniques were employed to identify and characterise the bacteria and the fungi isolated from detritus and decaying fronds.

4.2. Material and methods

Two fresh seaweed samples were obtained for use in the isolation of fungi, namely, *Fucus* was obtained from Robin Hood's Bay Yorkshire, UK and *Laminaria* from Filey North Yorkshire.UK (Fig.4.1).

4.2.1. Descriptions of sampling

The decaying fronds of *Fucus* and *Laminaria* were rinsed with distilled water to remove attached soil particles and other materials. Then placed in sterile transparent zip-lock bags and transported to the laboratory.

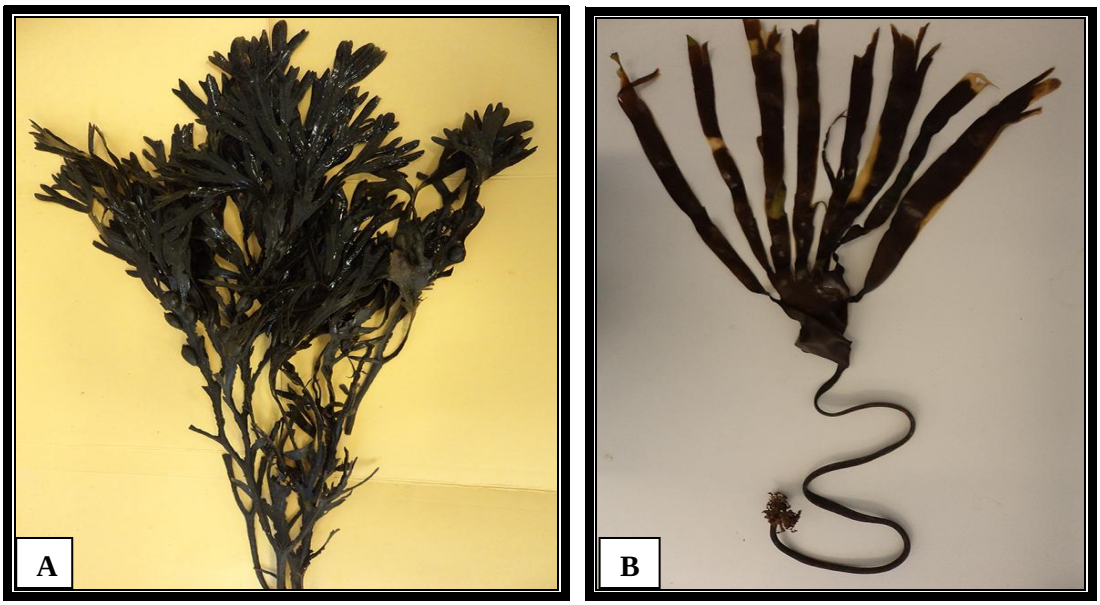


Figure 4.1: Fresh seaweed (A) *Fucus* and (B) *Laminaria.*

Several marine sand samples were collected and stored in polythene bags (20cm square) at 4 °C until used. Salinity of seawater was determined by the use of a laboratory Salinometer

(Fig. 4.2). The pH was also determined using a pH meter with a glass electrode (3310, Jenway Ltd, UK).

Figure 4.2: Salinometer.

4.2.2. Initial isolation of marine microorganisms

Isolation of microorganisms from both marine plants and marine sands samples was carried out using Boyd and Kohlmeyer medium (B and K) described by Verma (2011) and also using sodium alginate medium (APY). The media sterilised by autoclaving at 121°C for 15 minutes. In this study two types of marine microorganisms were isolated: marine fungi and marine bacteria.

4.2.2.1. Isolation of marine fungi

From decaying fronds of seaweed (*Fucus* and *Laminaria*); marine fungi were isolated by particle plating method using B and K medium prepared with sea water containing 10% antibiotic solution. Particle-plating method is a selective isolation technique described by Verma (2011). The decaying fronds of *Fucus* and *Laminaria* were cut into pieces (1 cm) under sterile conditions and then surface sterilized for 5 min with of 0.5% sodium hypochlorite solution. The solution was then drained and the frond pieces washed 3-4 times with sterile sea-water. These pieces were homogenized using a sterile mortar and pestle and passed through sterile sieves followed by washing with sterile sea-water. The particles retained on the sieve were then transformed on Boyd and Kohlmeyer (B and K) agar medium consisted of 1 g yeast extract, 2 g peptone, 10 g dextrose and 15 g agar prepared with 1000 ml of natural seawater and supplemented with 10% antibiotic solution to prevent bacterial growth.

4.2.2.2. Isolation of marine bacteria

Isolation of bacteria was carried out from marine sand using APY agar medium described by Sawabe *et al.*, (1995). The medium consists of 0.5g sodium alginate (Sigma), 0.1g tryptone, 0.1 g yeast extract, 15g agar (Oxoid No1), in 1000 ml natural sea water at pH 8.0. All components were dissolved and sterilised by autoclaving at 121°C for 15 minutes. Nystatin (Sigma) was added as solution to avoid fungal growth. The isolation of bacteria was carried out as follows: 25 g of marine sand were added to a flask containing 250 ml of sterile seawater. From 10^{-1} to 10^{-5} dilutions, 0.1 ml was inoculated on an APY agar medium and incubated at 37°C for 7 days. The colonies on the APY agar plates were purified on the same medium. Developed colonies were then, streaked onto new APY plates and incubated in order to obtain pure cultures. To ensure strain purity, the isolations were conducted in triplicate. The colonies were maintained on Tryptic soy agar (Fluka) consisted of; 17g casein, 3g soya peptone, 5g sodium chloride, 2.5 g glucose, 2.5 dipotassium hydrogen phosphate and 15 g agar in 1000 ml deionised water.

4.2.3. Assays for degradation of sodium alginate in plates

An experimental investigation was conducted to search for alginolytic microorganisms. Alginolytic microorganisms can use alginate as carbon sources for their growth and survival.

4.2.3.1. Degradation of sodium alginate in plates by fungi (alginolytic fungi)

Plate assays for alginate degradation of marine fungi (isolated from seaweed) were performed on APY agar plates. Four marine fungi isolated from the above methods were qualitatively screened for the presence of alginate degrading enzymes using model dye-release methods.

Table 4. 1: Composition of artificial seawater ;Lyman and Fleming formula (Kester *et al*., 1967).

Components	Amount/L
Sodium chloride (NaCl)	5 g (0.5%) and 30 g (3%).
Sodium sulphate (Na_2SO_4)	4.008 g.
Potassium chloride (KCl)	0.677g.
Sodium bicarbonate ($NaHCO_3$)	0.196 g.
Potassium bromide (KBr)	0.098 g.
Boric acid (H_3BO_3)	0.026 g.
Sodium fluoride (NaF)	0.003 g.
Magnesium chloride ($MgCl_2.6H_2O$)	1.071mg.
Mill-Q water	1000 ml.

Also, additional fungal isolates were tested; *Trichophyton mentagrophytes* (dermatophytic fungi), *Aspergillius oryza, Pencillium daleae,* unknown fungus (laboratory strains), *Aspergillius flavus, Penicillium verrclusium* and *Motriella amobia* (isolated from hair). The screening for the presence of alginate-degrading fungal enzymes was carried out on sodium alginate agar medium (APY) as described above in 1000 ml calcium- and strontium-free artificial sea water (NaPYNS) pH 7 (Table 4.1) described by Lyman and Fleming . NaPYNS was included two concentration of sodium chloride; 0.5% and 3% at pH 7.5. For this test; 8mm disc of each fungal culture was inoculated on plates containing the test medium, incubated at 25 °C for 2-3days. The presence of alginate lyase activity was detected by gently flooding plates with a solution of 0.05% (wt/vol) aqueous ruthenium red stain (Sigma). Plates were flooded with the dye solution and left at room temperature approximately 10 min. Regions which indicate degraded alginate by alginate lyses were recognized as light pink clearing zones against a dark red background (Gacesa and Wusteman, 1990).

4.2.3.2. Degradation of sodium alginate in plates by bacteria (alginolytic bacteria)

Eleven laboratory strains of bacteria isolated from the above methods were screened for the presence of alginate degrading enzymes using model dyes. In addition, extra laboratory isolates from G10 laboratory in Molecular Biology and Biotechnology Department were also tested:

- *Bacillus cereus, Bacillus thuringiensis* and *Bacillus megaterium.*

- *Pseudomonas aeruginosa* strain ATCC 27853, *Staphylococcus aureus, Escherichia coli, Cupriavidus necator* and *Rhizobium sp*

Medium

The presence of alginate-degrading bacteria enzymes was detected using APY medium in 1000 ml calcium- and strontium-free artificial sea water (NaPYNS, pH 7) include 0.5% and 3% sodium chloride at pH 7.5.

Preparation of turbidity standard suspension

The sensitivity tests were modified to $1.5x10^6$ colony forming units (CFU) which equals 0.5 McFarland. The colonies were inoculated into Falcon tubes containing nine ml of phosphate buffer pH 7. The turbidity was measured in spectrophotometer at 600 nm and the measurement was recorded. The tube was diluted by transferring 1 ml of the phosphate buffer to another tube containing 9 ml sterile phosphate buffer in order to create the first dilution (10^{-1}). 0.1 ml from different dilution was inculcated and spread on medium plates. The plates were incubated for 48hours. The plates were then flooded with a solution of 0.05% (wt/vol) aqueous ruthenium red stain and left at room temperature approximately 10 min. Light pink regions were recognized to confirm that the alginate had been degraded by alginate lyase.

4.2.4. Alginate lyase activity in broth medium

Detection of alginate lyase in earlier methods needs further assessment. In this study there are two approaches were done to assess alginate lyase activity:

- Release of alginase is associated with formation of new unsaturated non-reducing ends. Consequently, alginase activity was determined by measuring the increase in the absorbance at 235 nm (An *et al*., 2008).
- Alginase saccharifying activity was determined by measuring the increase in concentration of reducing sugar. The amount of reducing sugar formed was determined using 3, 5-dinitrosalicylic acid (DNS) method for reducing sugar with glucose as standard substrate. Alginase lyase was determined by measuring the increase in the absorbance at 540 nm using methods described by Tang *et al*., (2009).

4.2.4.1. Fungal alginate lyase activity

Nine species of fungi produced clear zones in APY medium. Two unknown fungi were selected for enzyme activity in liquid medium. The fungi were grown in broth medium (50 ml in 250 ml Erlenmeyer flasks) containing 1g sodium alginate, 0.1g peptone, 0.1g yeast

extract; in 1000 ml calcium- and strontium-free artificial sea water pH 7 include 0.5% sodium chloride. The mixture sterilized in Erlenmeyer flasks (250 ml).

- **Alginate lyase activity as unsaturated non-reducing ends**

Alginate lyase activity was determined by measuring the increase in the absorbance at 235 nm due to the formation of new unsaturated non-reducing ends. *Alternaria tenuissima* was selected for this test using the test medium at pH 7.4. For this test; 8mm disc of each fungal culture was inoculated in flasks. The medium incubated on revolving shakers (130 rpm) at 25°C for 3, 5, 10 and 12 days. A set of uninoculated flasks was run as control. The pH was measured during the incubation period by using a pH meter. The reaction mixture was composed of 2 ml of 0.05 M phosphate buffer (pH7.0), 0.5 ml 0.6% (w/v) sodium alginate and 0.5 ml of supernatant and 0.5 ml 0.1% sodium chloride. The reaction was incubated at 30 °C for 30 min. After the end of incubation period, the reaction mixture was transferred into quartz cuvette. The blank contained 2 ml from reaction mixture diluted with 5 ml distilled water. The alginase activity was expressed at 235 nm (An *et al.*, 2008).

- **Alginate lyase activity as reducing ends**

Also alginate lyase activity was determined by measuring the increase in the absorbance at 540 nm due to the formation of new reducing ends. *Dendraphiella salina* was selected for this test using the same medium at pH 5.5. The medium incubated on revolving shakers (130 rpm) at 25°C for 3, 5 and 7 days. A set of uninoculated flasks was run as control. The pH was measured during the incubation period by using a pH meter. The reaction mixture contained 100 µl of supernatant, 100 µl of substrate1.0% (w/v) sodium alginate. The mixture was incubated at 40 °C for 10 min. 400 µl of DNSA regent was added to the mixture and boiled for 15 min. 4.4 ml with deionized water was added and the colour was measured at 540 nm. The result was expressed as mg glucose/ ml. The alginate lyase activity was then determined by reference to standard curve of D-glucose at 540 nm prepared from a standard solution of D-glucose (Sigma) (see Appendix A).

4.2.4.2. Bacterial alginate lyase activity

Pseudomonas aeruginosa strain ATCC 27853 was used for quantitative enzyme estimation. The isolate was streaked on the alginate medium plate in order to confirm the homogeneity of the strain. A single colony from *Pseudomonas aeruginosa* was used for the alginate lyase activity. The bacterium was grown in (broth) medium (50 ml in 250 ml Erlenmeyer flasks) containing 1 g sodium alginate, 1g yeast extract, 2 g tryptone, 5 g sodium

chloride in 1000 ml distilled water at pH 7.6 (Kitamikado *et al*., 1990). The medium adjusted at pH 7.2. Alginate lyase activity was determined by measuring the increase in the absorbance at 235 after 16, 24, 48 and 96 hours. Also, the alginate lyase activity was measured at 540 nm after 12, 24 and 48 hours incubation at 37 °C in150 rpm using the same steps in fungi.

4.2.5. Chemical Analysis of degraded fresh seaweed *(Fucus and Laminaria*)

The chemical analysis aimed to detect some minerals from decaying seaweed. Samples from *Fucus* and *Laminaria* were analyzed for macronutrients (Nitrate and ammonium). The fronds of *Fucus* and *Laminaria* were cut into pieces (1 cm) and mixed with agricultural soil (Fig. 4.3).

Figure 4.3: Seaweed fronds after cutting. **(A)** *Laminaria* and (B) *Fucus*

4.2.5.1. Determination of nitrate in agricultural soil amended with *Fucus* and *Laminaria*

All soil samples (50g) were placed in polythene bags and amended with (5g *Fucus* and 5g *Laminaria)* and mixed thoroughly. The same methods described in the pectin chapter were done.

4.2.6. Chemical analysis of degraded commercial seaweed

This approach aimed to assess the amount of sulphur, nitrate and ammonium release from degraded seaweed using commercial seaweed (Fig.4.4).

Figure 4.4: Seaweed meal.

4.2.6. 1. Determination of the sulphur in agricultural soil amended with seaweed

The soil (50g) was placed in polythene bags and amended with 5g seaweed meal and mixed carefully. A control was set-up lacking without added seaweed. The modified soils were incubated in polythene bags, closed with small holes to allow for gas exchange. The bags were set up in triplicate and incubated for 28 days at 25°C. At zero time and at 7 days intervals samples were extracted. Soil samples (1g) were shaken with (10ml) of LiCl (0.1 M) for 15 min at 100 rpm using an orbital shaker and then the samples were filtrated through Whatman No.1 filter paper.

Determination of sulphate

The turbidimetric sulphate method was used to determine the oxidation of sulphur (Hesse, 1971). For this test, the same methods described in Chapter 2 were done.

4.2.6.2. Determination of nitrate and ammonium

The same method described in pectin using 5g of seaweed meal and 50g soil was done for this test.

Statistic analysis of data

All abservations were presented as Mean ± SE (Standard error). Sigma Plot© (Version12.0) was run to analyze data. $P < 0.05$ was considered as significant. Matching three samples t-test was performed to check whether there were significantly different.

4.2.7. Molecular identification techniques for bacteria and fungi

In recent years, several studies have focused on molecular techniques. They have demonstrated the accurate identification and classification of microbes.

4.2.7.1. Genomic DNA extraction for bacteria and fungi

Firstly, genomic DNA was isolated from each strain using (Key prep- Bacterial DNA Extraction Kit) (ANACHEM, labstore, UK) by following procedures approved for bacteria; and using (Norgen Fungi Genomic DNA Isolation Kit) (GENEFLOW LIMITED, Labstore, UK) by following procedures approved for fungi. Secondly, PCR was performed using suitable primers to produce 16S rRNA or fungal ITS gene. The genomic DNA was separated by gel electrophoresis on 1% agarose to check for purity. Fungal genomic DNA was isolated from four strains (F1, L4, L5 and L6) grown in alginate broth medium using (Norgen Fungi Genomic DNA Isolation Kit) and was performed by the same procedure described in keratin chapter. In addition, bacterial genomic DNA for terrestrial and marine bacteria was done using the same procedure in pectin Chapter with small modifications (nutrient broth medium for terrestrial bacteria and alginate broth medium for marine bacteria). Polymerase chain reaction (PCR) amplification was achieved using the same methods described in Chapter 2 and 3.

4.2.7.2. Phylogenetic analysis

The samples were immediately sent to the Medical School Core Genetics Unit for sequencing using ITS1/ITS4 and 16S FOR/16S REV. as sequencing primers. Fungal gene and 16S rRNA gene sequences were compared in The Basic Local Alignment Search Tool (BLAST). All sequences were corrected by the Finch TV software to identify matches with existing characterized sequences.

4.3. Results and Discussion

4.3.1. Measurement of pH and salinity of seawater

Based on the measurement of seawater alkalinity, the pH of seawater was pH 7.8. The current data demonstrated that pH range was optimum for the physical conditions of microorganisms. In addition, the salinity was approximately 34.2 ppt (parts per thousand). It can be clearly seen that the salinity is associated with the concentration of sodium chloride in seawater.

4.3.2. Growth of marine fungi isolated from seaweed plants

Fungi isolated from *Fucus* and *Laminaria* were able to grow on B and K media, which in sodium chloride is high. Growth of nine isolates of marine fungi were obtained in plates using seawater. The growth also occurred when the hyphae were transferred from B and K medium to Potato Dextrose Agar medium (PDA). The finding highlights that isolated marine fungi are therefore facultative and can adapt to growth on nutrient agar medium. This result is in agreement with the findings reported by Verma (2011).

4.3.3. Growth of marine bacteria isolated from marine sand

Eleven isolates of marine bacteria were obtained in alginate plates using seawater. The bacteria grew well on sodium alginate medium. Seven isolates showed very weak growth on sodium alginate medium and did not grow when they transferred in nutrient agar medium, while, the seven isolates grew well on Tryptic soy agar medium. However, out of eleven isolates, four isolates grew in all media. Overall, most marine bacteria could not adapt to grow on nutrient agar. Furthermore, they showed slow growth on alginate medium.

4.3.4. Alginolytic assay in solid media

The degradation rate of alginate was achieved within 2 days at 25°C by fungi and at 37°C by bacteria. Alginase production by microorganisms was determined in the presence of sodium chloride 3% and 0.5%.

4.3.4.1. Alginolytic assay of fungi

Most alginolytic fungi can use alginate as source of carbon. The current data showed that a small number of fungal isolates were able to exhibit enzyme activity (i.e. a clear zone) using artificial seawater 0.5% NaCl. The enzyme activity of fungal species is shown in Figure 4.5. Clearly, the isolates obtained from *Laminaria* (*Penicillium chrysogenum, Penicillium crustosum* and *Dendraphiella salina*) were more actively alginolytic than those isolated from *Fucus* (*Aspergillus sp*.) which did not produce the clear zones in the test medium. Terrestrial fungi; *Alternaria tenuissima*, *Trichophyton mentagrophytes, Penicillium verruculosum , Penicillium oryza, Penicillium daleae* and *Aspergillius flavus* showed slow alginate degradation within 2 days and only produced small number of clear zones. However, *Acremonium strictum, Motrilla amobia* showed weak growth but did not produce the clear zones. The results of the present study suggest that all fungi (marine or terrestrial) could not breakdown alginate using artificial seawater 3% NaCl. Among the plausible explanation for

these findings is that several species of marine fungi included *D. salina* do not need seawater and their growth were better at lower concentration of salts (low salinity) (Dela Cruz *et al*., 2006).

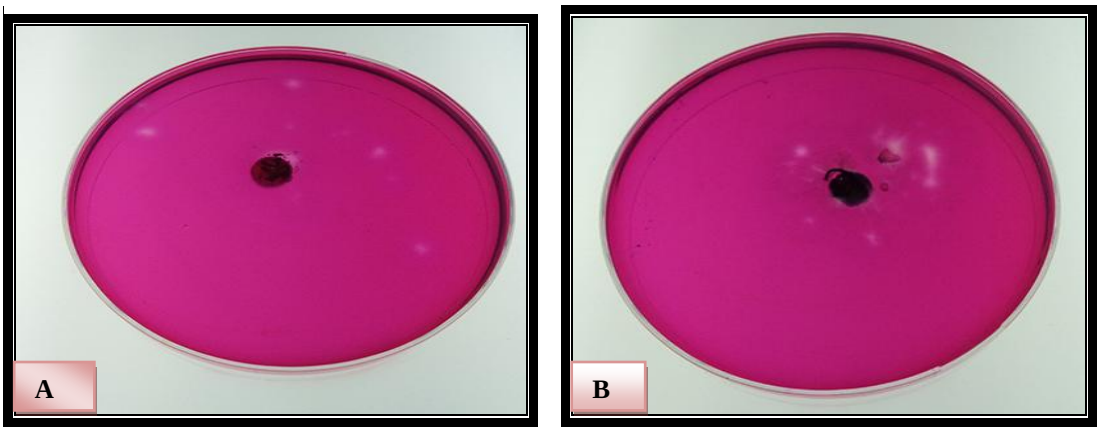


Figure 4.5*:* The effect of absence of carbon source after 48hours on the growth of alginolytic fungi. (A) *Penicillium crustosum* and (B) *Alternaria tenuissima.* All isolates grown on alginate medium 0.5% for 48 hours.

4.3.4.2. Alginolytic assay of bacteria

The use of sodium alginate allowed for the evaluation of alginate lyase activity by bacterial isolates. All marine bacteria and some terrestrial bacteria particularly; *P. aeruginosa* , *C.necator*, *B. megaterium* and *Rhizobium sp.* were capable of using alginate as a source of carbon (Table 4.2) because the absence of carbon source motivates the bacteria to digest alginate as carbon source leading to increased degradation. However, *B. cereus*, *Staph. aureus*, *B.thuringiensis* and *E.coli* did not produce a clear zone; this reflects the lack of extracellular alginate lyase in this terrestrial bacteria.

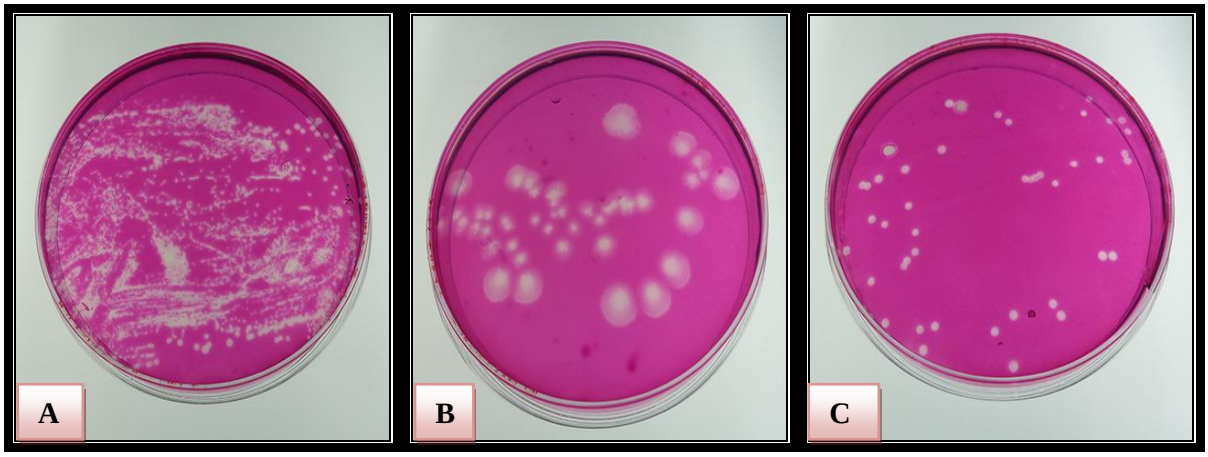


Figure 4.6: Comparison of detection of alginolytic bacteria in sodium alginate agar plates after 48hours incubation. (A) *Planococcus donghaensis* (B) *Bacillus stratosphericus.* (C) *Planococcus sp.* All strains were grown on alginate medium 0.5% for 48 hours.

Table 4.2: Enzyme activities of terrestrial bacteria after 48hours in alginate medium 0.5%.

Bacteria	**Alginate lyase activity**	**Bacteria**	**Alginate lyase activity**
E.coli	Negative	*C.necator*	Positive
B. cereus	Negative	*B. megaterium*	Positive
Staph. aureus	Negative	*Rhizobium sp.*	Positive
B.thuringiensis	Negative	*P. aeruginosa*	Positive

Interestingly, all marine bacteria were seen to be capable of alginate degradation. This might be due to the fact that alginate medium is considered to be a selective medium for these isolates (Table 4.3). Figure 4.6 shows high alginate lyase activity from marine bacteria. The most obvious finding to emerge from this study is that both marine and terrestrial bacteria are capable of breaking down sodium alginate. Alginate lyase production in alginate medium can be regulated via specific mechanisms.

Table 4.3: Enzyme activities of marine bacteria after 48hours in alginate medium 0.5%.

Marine Bacteria	Alginate lyase activity	Marine Bacteria	Alginate lyase activity
Bacillus licheniformis	Positive	*Bacillus stratosphericus*	Positive
Planococcus sp.	Positive	*Planococcus psychrotoleratus*	Positive
Planococcus donghaensis	Positive	*Planococcus crocinus*	Positive
Planococcus sp.	Positive		

It can be clearly seen that all bacteria (marine or terrestrial) did not breakdown alginate in the presence of artificial seawater 3% NaCl; salinity is therefore not essential alginate breakdown by fungi such as *D. Salina*.

4.3.5. Degradation of sodium alginate in broth medium

The action of alginate lyase was measure as the breakdown of glycoside linkages of alginate, leading to the production of reducing sugars (absorbed at 540 nm) and the release of unsaturated sugars (absorbed at 235 nm).

4.3.5.1. Alginate lyase activity from fungal strains

- **Alginate lyase activity as reducing ends**

In order to confirm that *D. Salina* is alginolytic fungi, the following experiment was carried out in liquid medium in the presence of sodium alginate. Under the experimental conditions used, *D. Salina* was able to grow in the liquid medium containing sodium alginate. Hydrolysis of alginate was achieved within 7 days at 25°C. The results from 3 to 7 days are shown in Figure 4.7.

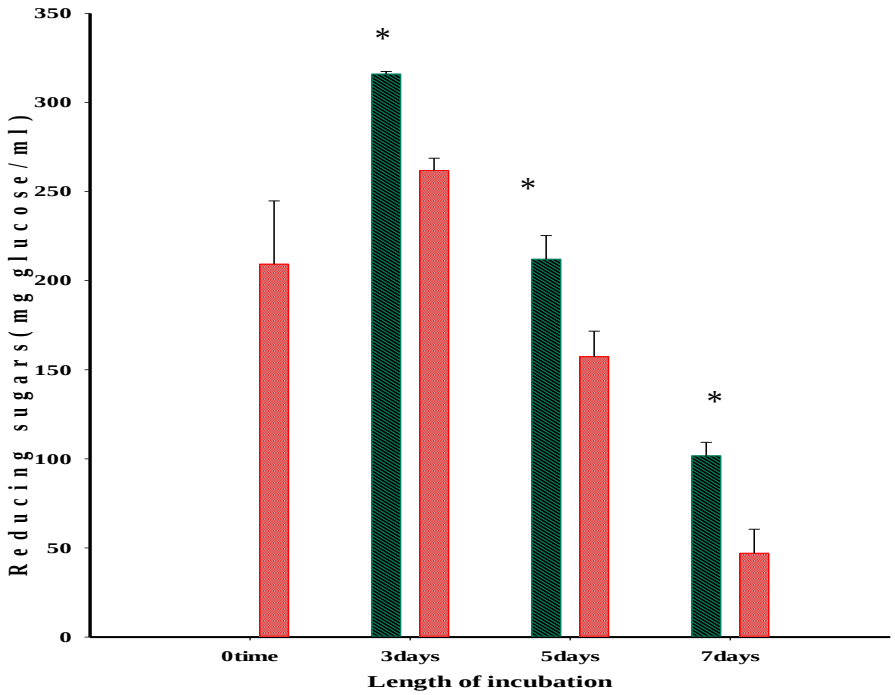


Figure 4.7: Release of reducing sugars by *D. salina* into alginate medium after 7 days incubation. *D. salina* . ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

It can clearly seen that *D. salina* shows increases reducing sugars (glucose) to the highest value after 3 days incubation, reducing sugars produced by *D. salina* then decreased after 5 and 7 days incubation. The finding is consistent with the work reported by Schaumann and Weide, (1995) who also found that the production of reducing substances from the degradation of sodium alginate by *D. salina* increased in early stages of incubation. Overall, the results show that *D. salina* grew well on sodium alginate as carbon. In addition, the finding provide evidence that sodium alginate is definitely degraded by *D. salina.* The present finding also support those reported by Shimokawa *et al*., (1997) who concluded that *D. salina* is an active alginolytic fungus. A change in the pH of the medium was noted after 3 days (Fig.4.8) showing that the degradation of sodium alginate was accompanied by alkalization of the medium. A slight change in pH was measured in the control while the fungal sample recorded a significant change in pH. A maximum pH value was 8.4 was recorded after 7 days incubation.

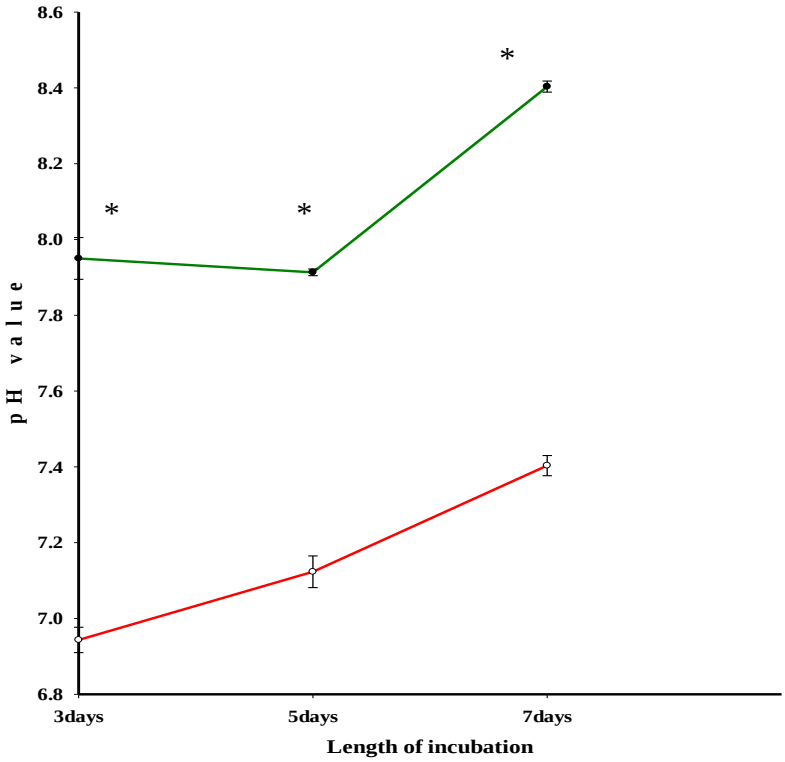


Figure 4.8: pH values of *D. salina* during incubation period. *Dendraphiella salina* (—■—) and control (—□—). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

- **Alginate lyase activity as unsaturated non-reducing ends**

Alginate lyase production from *Alternaria tenuissima* was also investigated. The strain was grown in sodium alginate medium and alginate lyase was determined by measuring unsaturated sugars. In Figure 4.9, the results show that the concentration of unsaturated sugars increased gradually at 3, 5 and 10 days then this value decreased at 12 days. It can be clearly see that the maximum amount of unsaturated sugars produced by *Alternaria tenuissima* occurred after 10 days of incubation. During all experimental stages, the activity of alginate lyase was observed. The most striking result to emerge from this work is *Alternaria tenuissima* has alginolytic potential a fact which has not been previously reported.

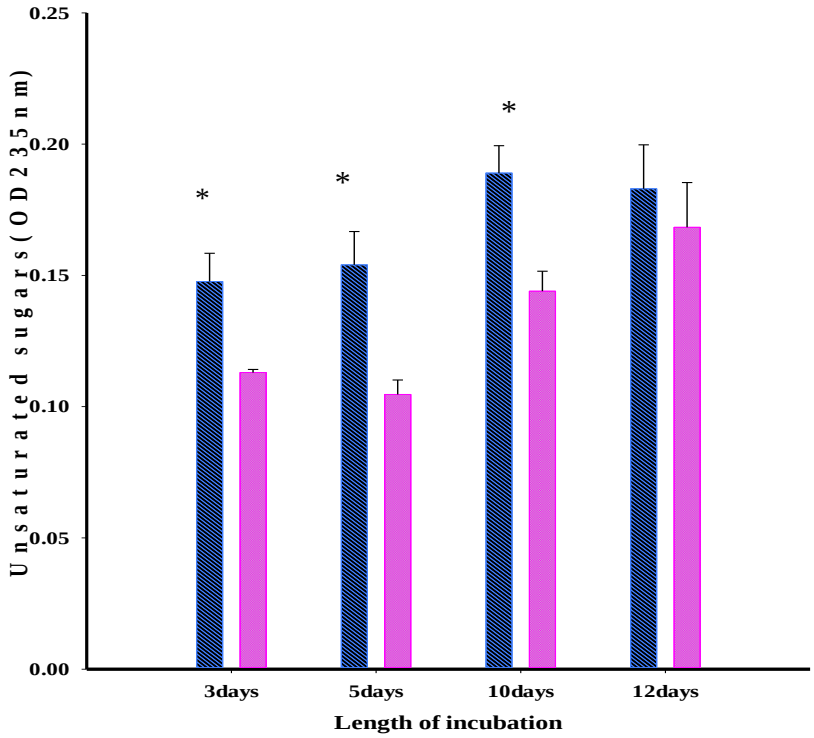


Figure 4.9: Release of unsaturated sugars by *Alternaria tenuissima* into alginate medium after 12 days of incubation. *Alternaria tenuissima* (▬) and control (▬). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

Figure 4.10 shows the change in pH rate in alginate medium inoculated by *Alternaria tenuissima.* It shows clearly that pH rate increased sharply after 3 days and the highest value was found at 10 days.

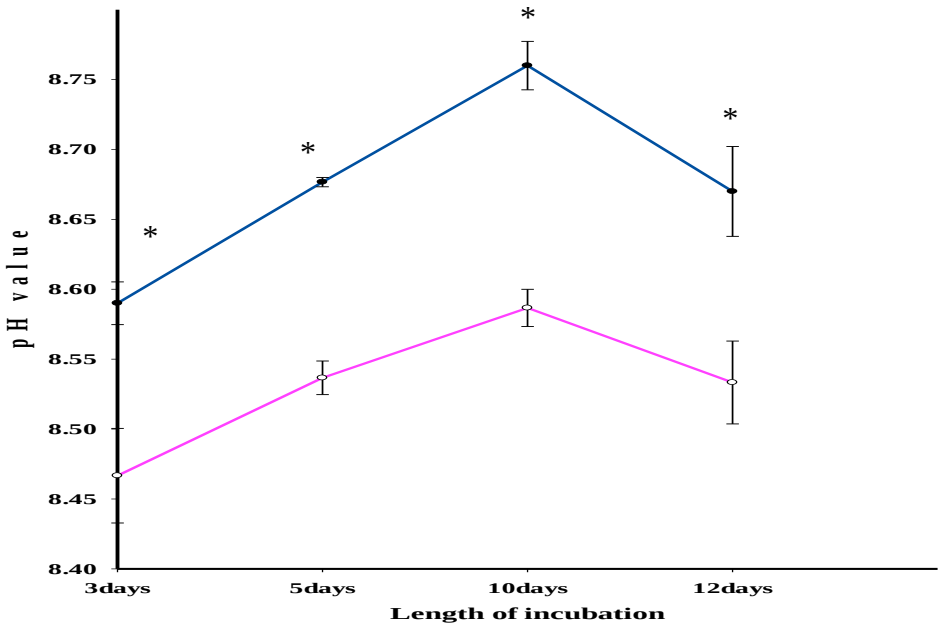


Figure 4.10: pH values of *Alternaria tenuissima* during incubation period. *Alternaria tenuissima* (—■—) and control (—○—). Means of triplicates (±) standard error.

*significant difference from the control (P <0.05).

The data reported confirm that the pH was associated with the degree of release of alginase, and that suggest that there is a relationship existed between the enzyme yield and increase of pH value.

4.3.5.2. Bacterial alginate lyase activity

The objective of this study was to determine whether the bacteria exhibit alginolytic activity.

- **Alginate lyase activity as unsaturated non-reducing ends**

Hydrolysis rate of alginate occurred within 96 hours at 37°C (Fig. 4.11.). The action of alginate lyase produced unsaturated oligomeric substrates (oligosaccharides). Unsaturated sugars released by *P. aeruginosa* increased gradually for the periods of 16, 24 and 48 hours.

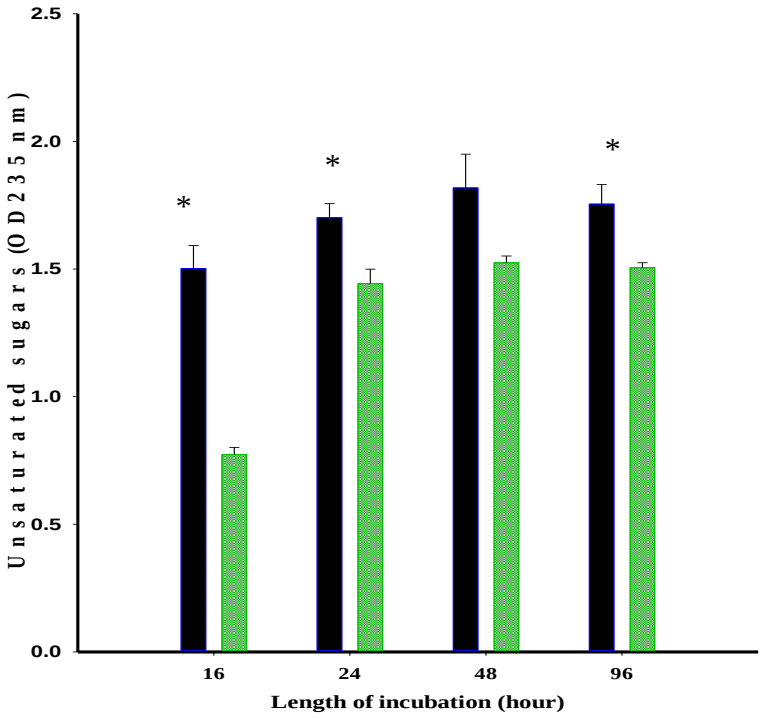


Figure 4.11: Release of unsaturated sugars by *P. aeruginosa* into alginate medium. *P. aeruginosa* (▬) and control (▬). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

The highest alginase production occurred after 48 hours, following which there was a moderate decrease. Unsaturated products increased with increasing incubation time. These results clearly show that alginate was degraded a finding consistent with the work of Linker and Evans, (1984). These authors reported that oligosaccharides (disaccharide, trisaccharide, tetrasaccharide and pentasaccharide) released by *P. aeruginosa* enzyme were not the final products and there was further degradation of oligosaccharides by this bacteria. Other workers have also found that the bacterium degraded the pentasaccharide to trisaccharide but the disaccharide is resistant to *P. aeruginosa* action. The above findings contradict the study by Boyd and Chakrabarty,(1994) who found that all tested strains of *P. aeruginosa* showed low levels of alginase activity when the activity measured as unsaturated sugar products. One of the more significant finding to emerge from this study is that alginase production by *P. aeruginosa* might be related to its pathogenicity. Such a correlation has been confirmed by Linker and Evans, (1984).

A slight change in pH was measured in the control while the bacterial sample recorded a significant change in pH, with a maximum pH value of 9.4 being seen after 48hours of

incubation (Fig. 4.12). This finding confirms that there is likely relationship between the production of alginase and alkalization of the medium.

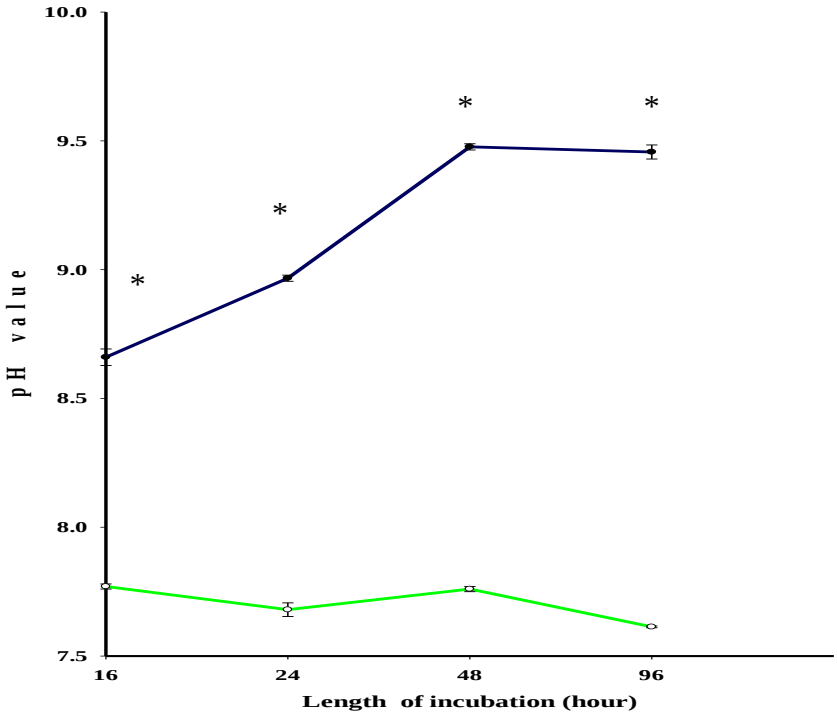


Figure 4.12: pH ranges produced by *P. aeruginosa* during incubation period. *P. aeruginosa* ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

- **Alginate lyase activity as reducing ends**

The digestion of alginate was also measured as reducing sugars at 540nm; the levels of reducing sugars produced by *P. aeruginosa* are shown in Figure 4.13. The rate of degradation increased rapidly after 12 and 24 hours. However, there was a slight decrease after 48 hours.

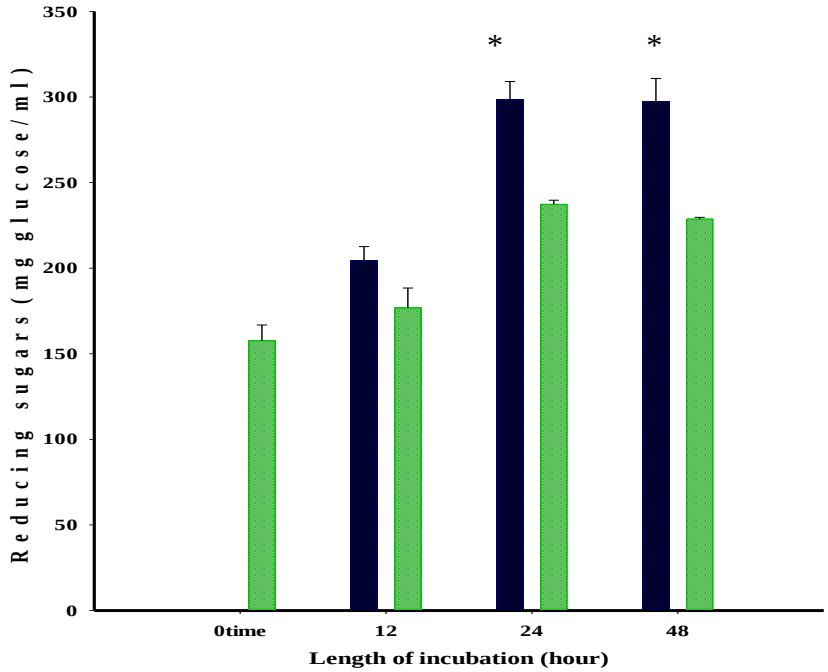


Figure 4.13: Release of reducing sugars by *P. aeruginosa* into alginate medium. *P. aeruginosa* ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

A rapid change in pH range was occurred in this experiment (Fig. 4.14). The pH increased over the incubation period and reached the highest 9.2 after 48 hours.

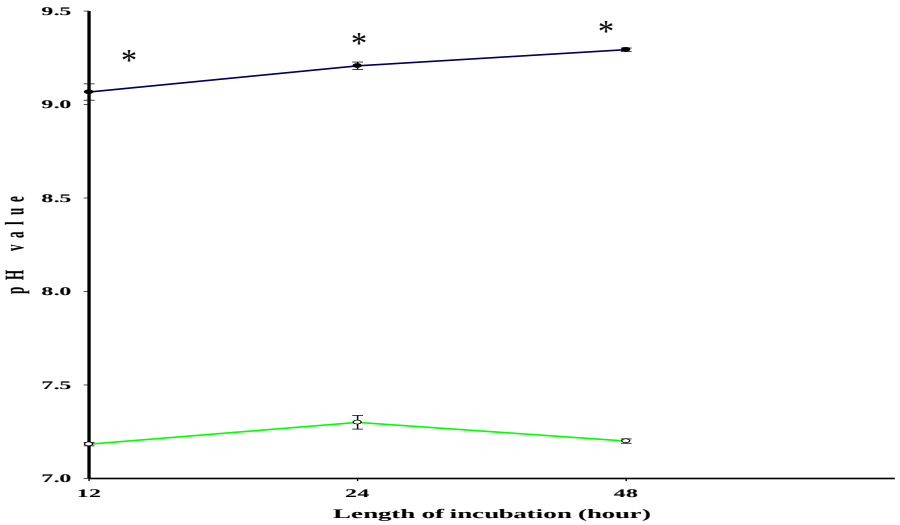


Figure 4.14: pH range produced by *P.aeruginosa* during incubation period. Means of triplicates (±) standard error. *P. aeruginosa* ( ) and control ( ).*significant difference from the control (P <0.05).

4.3.6. Chemical Analysis of degraded seaweed

Under favorable conditions, materials such as seaweed when added to the soil undergo rapid degradation which potentially supplies nutrients for both plant and microbial growth.

4.3.6.1. Net nitrate production from soil amended with *Fucus* and *Laminaria* (fresh seaweed)

The results in Figure 4.15 show the oxidation of ammonium to nitrate in agricultural soil amended with *Laminaria* and the control over a four week incubation period. There was an increase in the amount of nitrate production from the oxidation of ammonium throughout most of the incubation period in the treated soil. However, the levels of nitrate decreased after 4 weeks incubation which may be due to acidification. Levels of nitrate production found in amended soil are clearly higher than those found in the control.

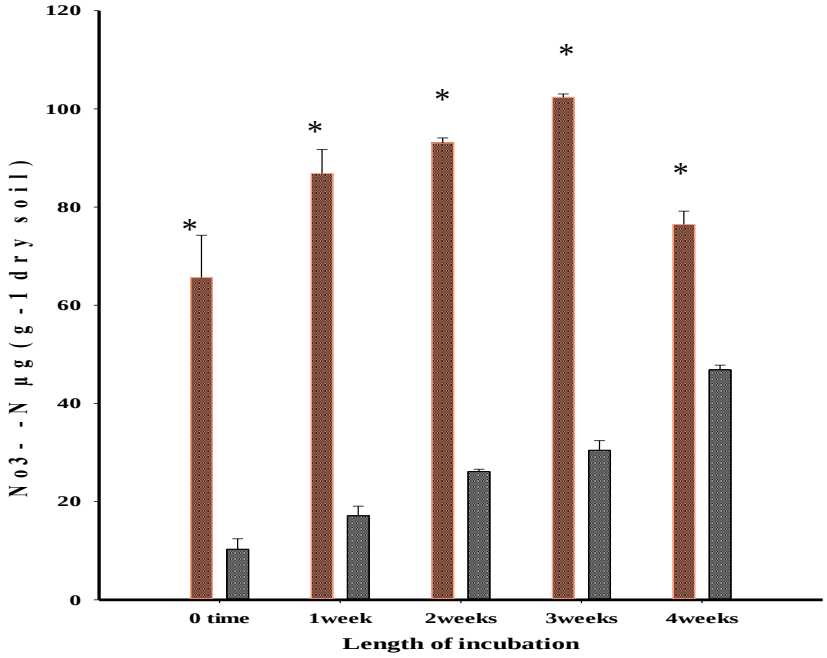


Figure 4.15: Nitrate production from the oxidation of ammonium in soil amended with *Laminaria.* Treatment ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

Figure 4.16 shows the oxidation of ammonium to nitrate in agricultural soil amended with the seaweed *Fucus.* The amount of nitrate production increased gradually at week 1 and 2 then decreased. There was also a slight rise in nitrate concentration in the control after 28 days. This study provides evidence that much higher concentration on nitrate was found in

Laminaria. This observation suggests that the microorganisms are extremely active in *Laminaria* nitrification.

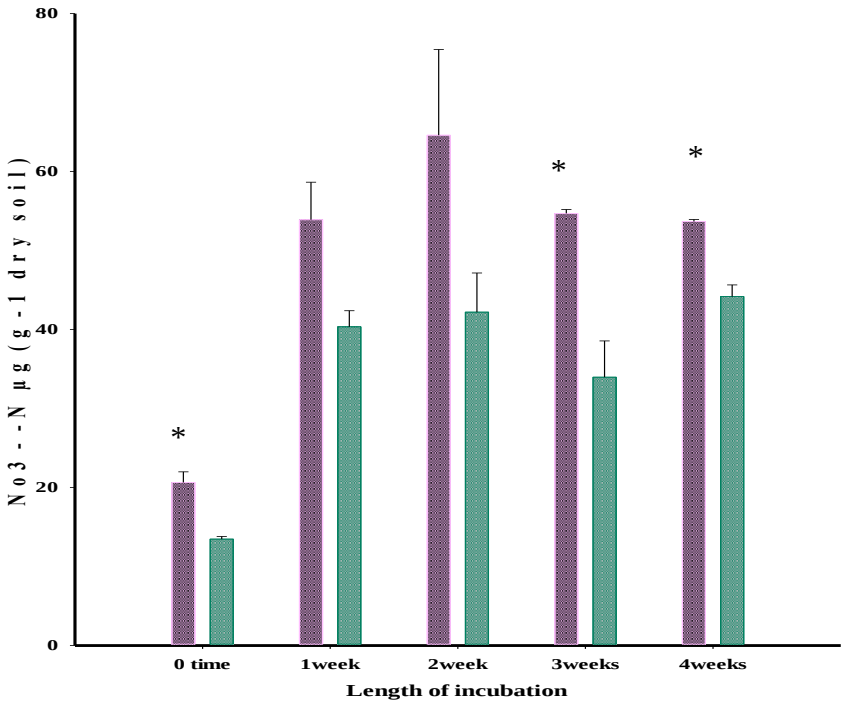


Figure 4.16: Nitrate production from the oxidation of ammonium in soil amended with *Fucus.* Treatment ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

4.3.6.2. Ammonium production from soil amended with *Fucus* and *Laminaria*

Figure 4.17 and 4.18 show a comparison in ammonium production between *Fucus* and *Laminaria* over the four week incubation period.

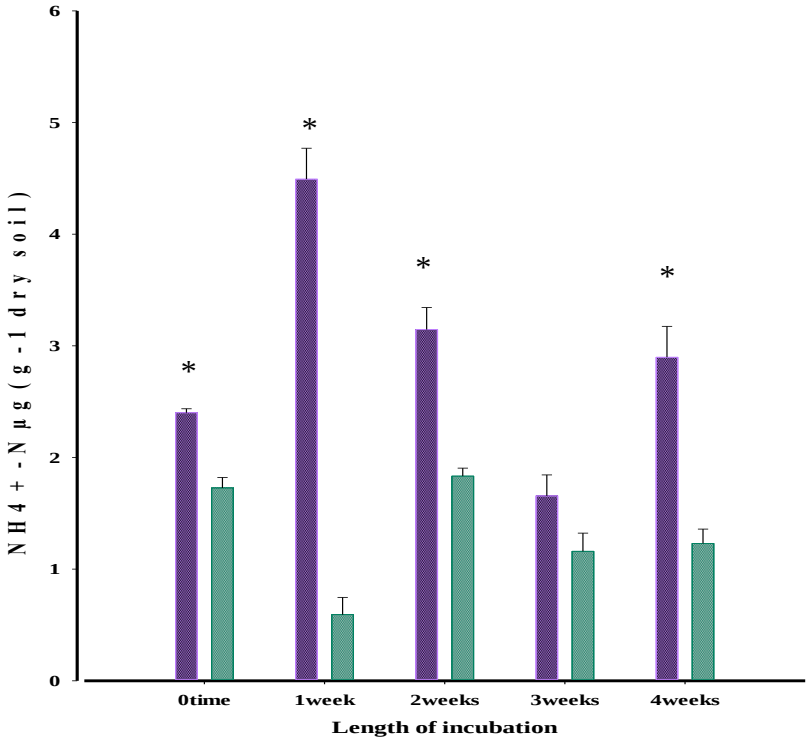


Figure 4.17: Ammonium production from the hydrolysis of *Fucus.* Treatment ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

The level of ammonium was considerably increased after 7 days in *Fucus* and that the highest ammonium production was found at week 1 (Fig. 4.17). The result obtained from *Laminaria* clearly show that there was a gradual increase in ammonium production from the hydrolysis of *Laminaria* throughout the whole treatment period and that ammonium production peaked at 21 days.

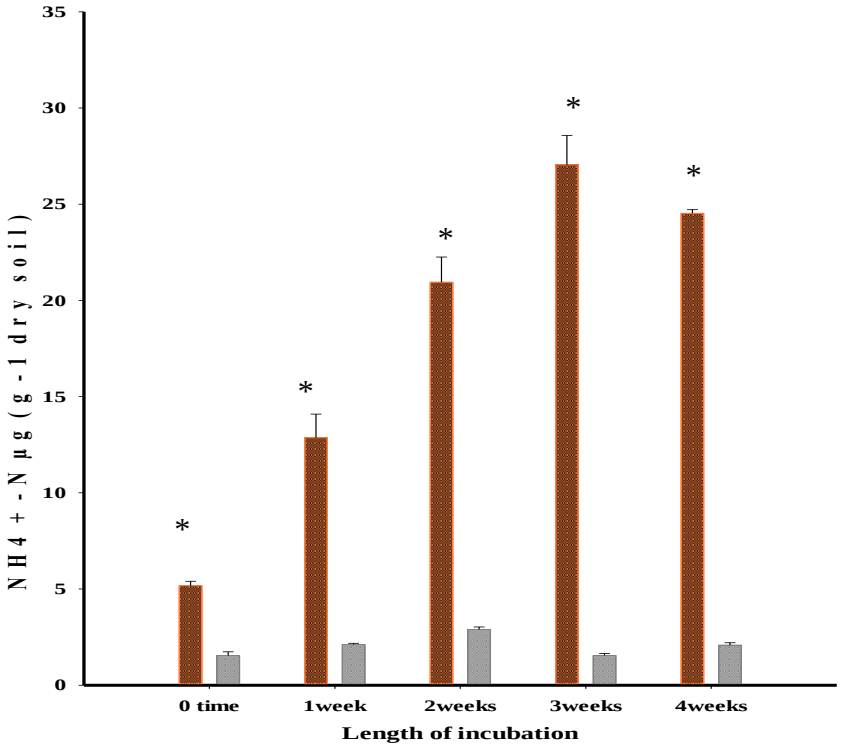


Figure 4.18: Ammonium production from the hydrolysis of *Laminaria.* Treatment ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

4.3.7. Chemical Analysis of degraded commercial seaweed (seaweed meal)

The breakdown of commercial seaweed was also tested because of its use as a fertilizer and soil conditioner.

4.3.7.1 Sulphate production in agricultural soil amended with seaweed meal

Figure 4.19 shows the oxidation of sulphur in agricultural soil amended with seaweed over four weeks incubation period. The results show that microbial S-oxidation of sulphur increased from day 7 and continued increasing throughout the 21 days incubation period while there was slight rise in sulphate concentration in the control after 28 days. It can be clearly seen that oxidation of sulphur reached a peak of week 3.

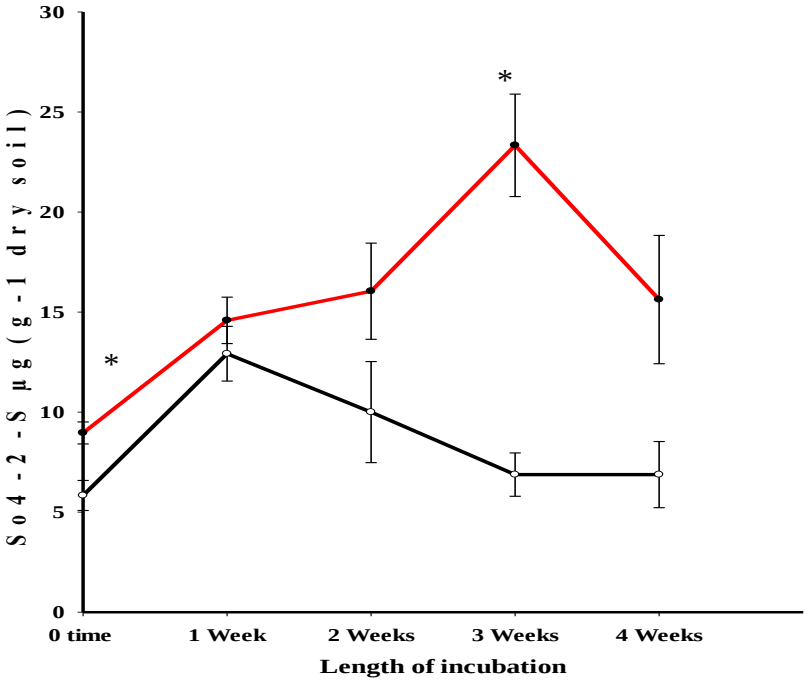


Figure 4.19: Sulphate production from oxidation of elemental sulphur in soil amended with seaweed meal. Treatment (—●—) and control (—○—). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

4.3.7.2. Determination of nitrate and ammonium in agricultural soil amended with seaweed meal

The results given in Figure 4.20 and 4.21 show the amount of nitrate and ammonium in soil amended with seaweed. Figure 4.20 clearly shows that the amount of ammonium increased gradually and that the highest ammonium production occurred at week 2 of the incubation period and then decreased after 3 and 4 weeks. These results suggest that dry seaweed can be favourable substrates for microorganisms and that seaweed meal undergoes microbial degradation in the soil leading to the release of organic substrates which are then returned into the ecosystem.

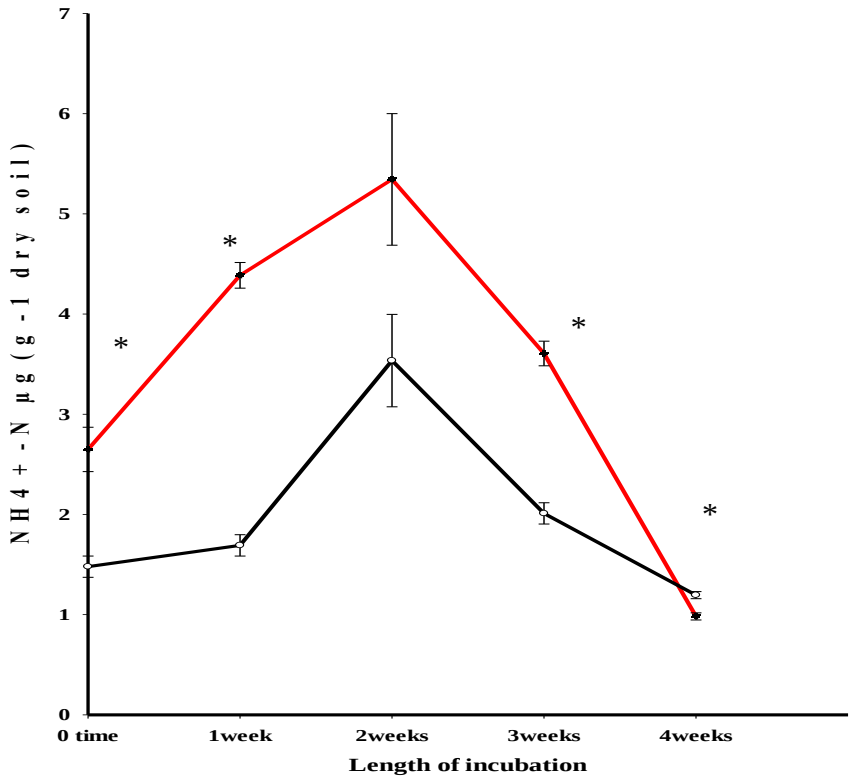


Figure 4.20: Ammonium production from the hydrolysis of seaweed. Treatment ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

The concentration of nitrate was lower than that of ammonium (Fig.4.21). Rapid nitrate production occurred, and then this level decreased sharply and remained stable.

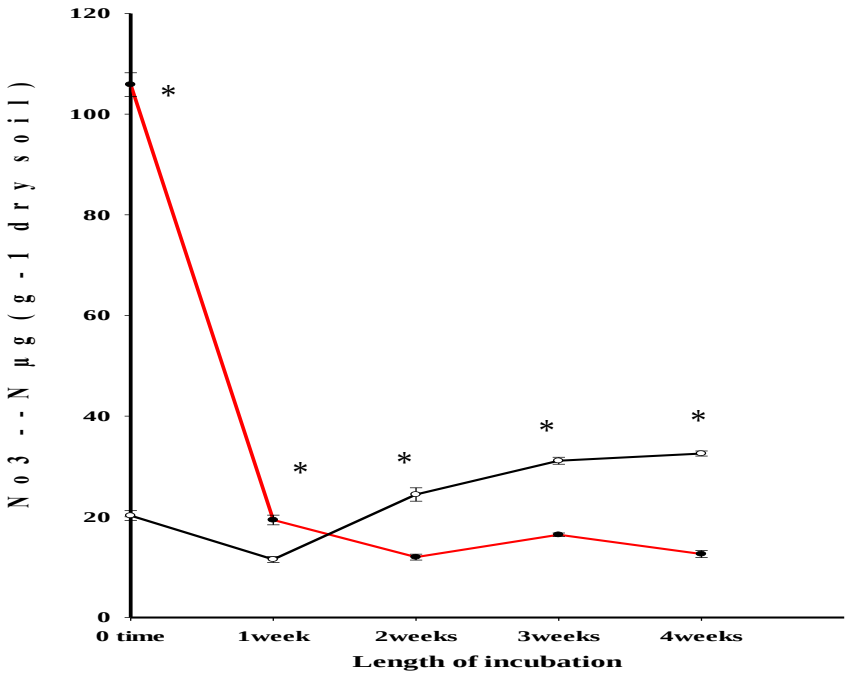


Figure 4.21: Nitrate production from the oxidation of ammonium in the soil amended with seaweed meal. Treatment (—●—) and control (—○—). Means of triplicates (±) standard error. *significant difference from the control (P <0.05).

4.3.8. Molecular identification techniques for bacteria and fungi isolated from marine environment

4.3.8.1. Extraction of genomic DNA

In this study, eleven bacterial strains have been isolated from marine sand. The whole genomic DNA was successfully extracted from eleven strains using (Key prep- Bacterial DNA Extraction Kit). DNA Hyperladder 1 has been used in these studies to determine the size of DNA molecules. Also, four fungi were isolated from *Fucus* and *Laminaria* and their genomic DNA were extracted successfully.

4.3.8.2. PCR amplification of extracted DNA

The DNA sequence gene of 16S rRNA was determined for bacteria and the DNA sequence gene of fungal gene has been determined for four strains. Amplified 16S rRNA and fungal genes are show in Figure 4.22. PCR products were observed in the most of lanes.

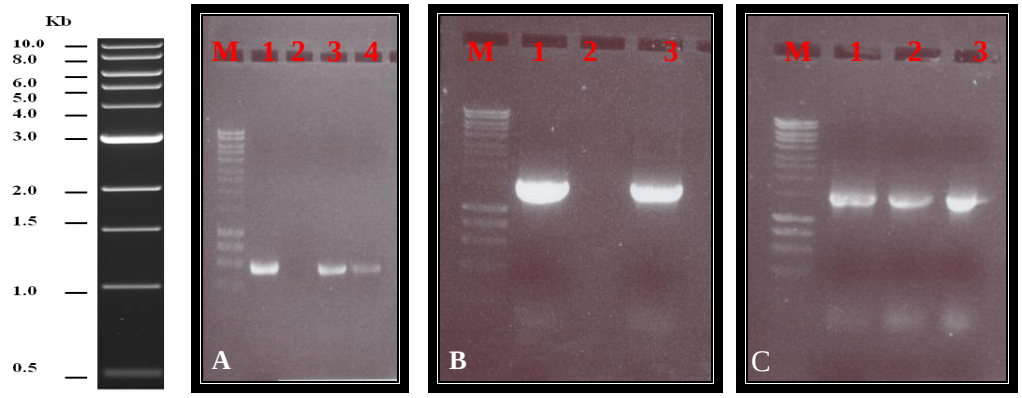


Figure 4.22: PCR product of marine isolates (A) Lane 1: *Aspergillus sp.*,Lane 3: *Penicillium crustosum* and Lane 4: *Dendraphiella salina* (B) Lane 1: *Bacillus pumilus* and Lane 3: *Bacillus areophilus* (C) Lane 1: *Bacillus altitudinis*, Lane 2*: Bacillus* sp. and Lane 3: *Bacillus stratosphericus* .

4.3.8.3. Phylogenetic identification of unknown bacteria and fungi

The sequences data were used to produce a phylogenetic tree providing the basis for efficient phylogenetic investigation of each microorganism. Table 4.4 shows 16S rRNA sequence and Table 4.5 shows fungal analyses representing the closest matches of all strains.

Table 4.4: Summary of 16S rRNA sequence analyses of bacteria cultured from marine sand.

Sample	Representative sequence	Closest matches Identification	Sequence Identity	Length of Sequence (bp)	NBCI (Accession number)
From marine sand	MB1	*Bacillus licheniformis*	98%	1083	JX847117.1
	MB2	*Planococcus sp.*OR1	99%	1555	JF742665.1
	MB3	*Planococcus donghaensis*	98%	1452	NR044073.1
	MB4	*Planococcus sp.*SS1.3	99%	1511	KC160837.1
	MB6	*Planococcus psychrotoleratus*	98%	1502	AF324659.1
	S1	*Bacillus pumilus*	97%	1512	GQ903427.1
	S2	*Bacillus areophilus*	98%	1513	JX680140.1
	ALG2	*Bacillus altitudinis*	97%	1453	KC172064.1
	ALG3	*Bacillus* sp.	99%	1485	KF010630.1

Table 4.5: Summary of identity of fungal sequence of marine fungi cultured from *Laminaria.*

Sample	Representative sequence	Closest matches Identification	Sequence Identity	Length of Sequence (bp)	NBCl (Accession number)
From *laminaria*	L4	*Penicillium chrysogenum*	99%	580	JX139706.1
	L5	*Penicillium crustosum*	99%	540	JX869585.1
	L6	*Dendraphiella salina*	99%	583	DQ411540.1

All strain sequences were compared with other sequences in the database and the varying percent of identification-level is shown . High identity was recorded from *Penicillium chrysogenum, Penicillium crustosum, Dendraphiella salina, Bacillus* sp., *Planococcus sp*. (99%). Meanwhile, a 98% identity was shared between *Planococcus donghaensis, Planococcus psychrotoleratus* and *Bacillus areophilus.* Sequence analysis showed that 583 nucleotide fragment was obtained from *L6* (Fig.4.23); the sequence was the same *Dendryphiella salina* sequence.

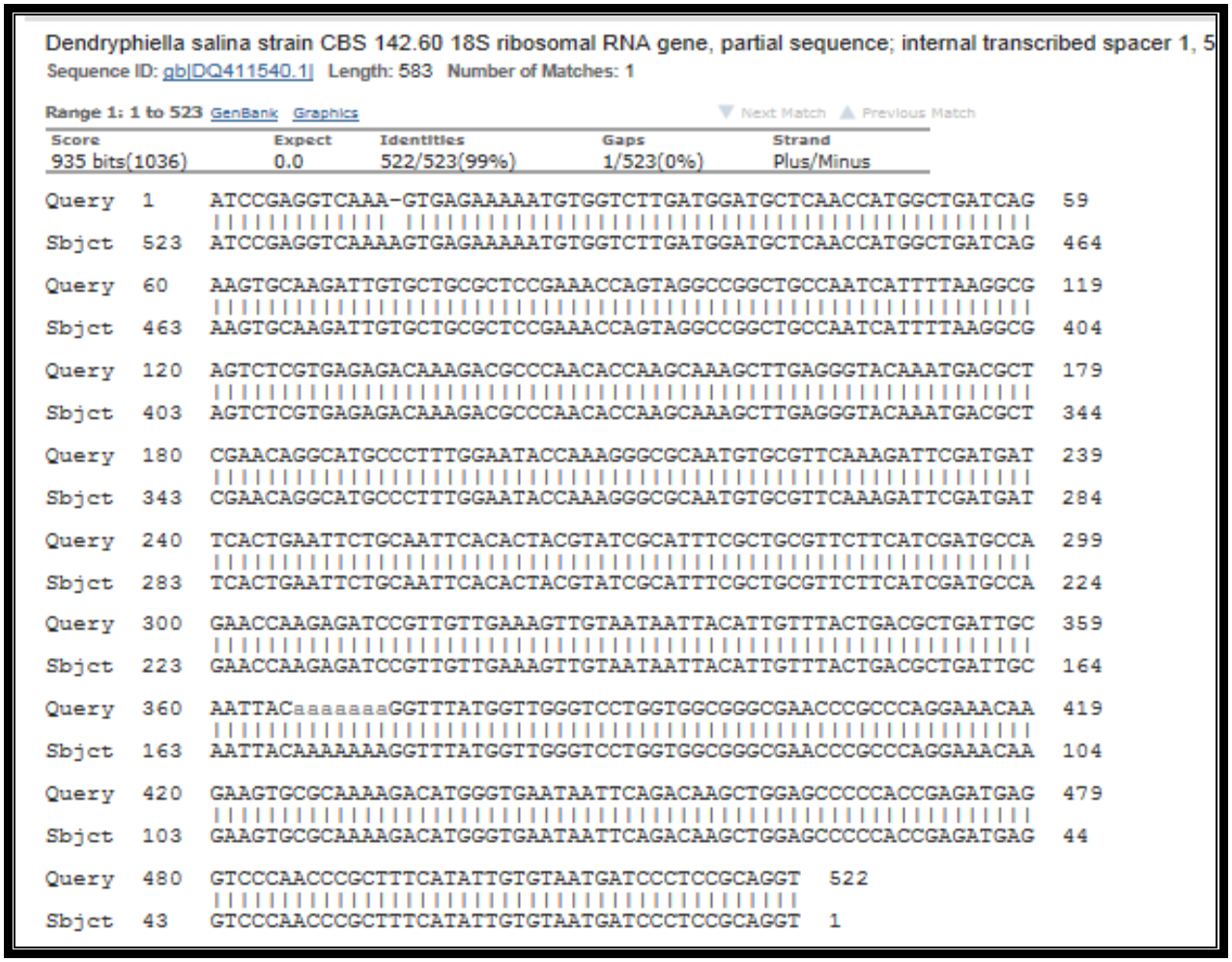


Figure 4.23: Nucleotide **s**equence of the marine fungus *Dendryphiella salina.*

The root of tree obtained from *Dendryphiella salina* is shown in Figure 4.24 two major branches can be seen, the one containing *Dendryphiella salina* has two branches indicating an ascomycete and fungal endophyte.

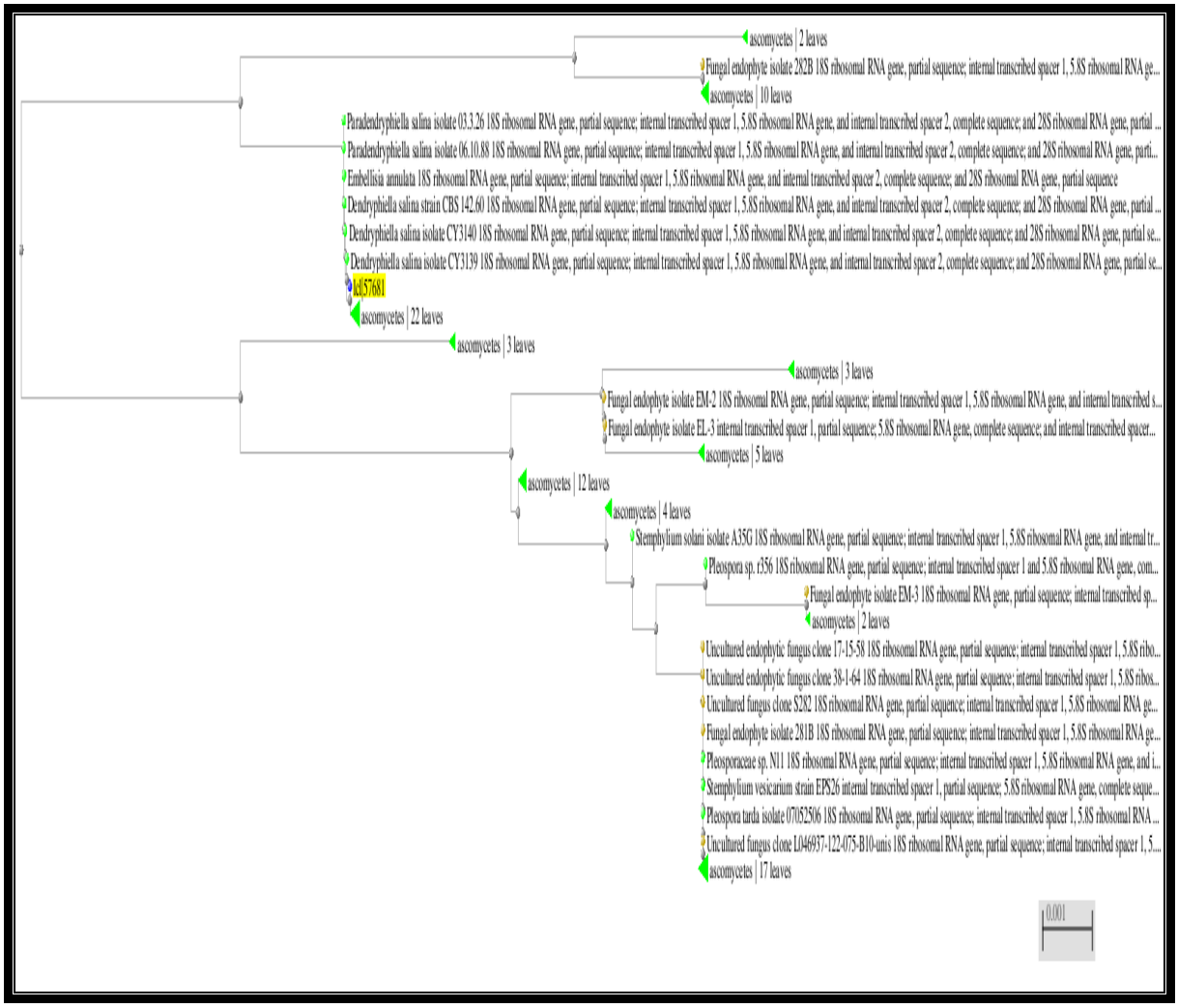


Figure 4.24: The tree of the marine fungus (*Dendryphiella salina*).

CHAPTER 5

CHAPTER 5
STUDIES ON MICROBIAL DEGRADATION OF CHITIN

5.1. Introduction

There has been an increasing interest in the clean up the marine environments from chitin wastes such as shells of crustaceans and recent developments in chitin degradation using chemical solutions have heightened the need for friendly environmental treatments deal with these wastes.

5.1.1. Properties of chitin

After cellulose, chitin is considered the second most abundant carbohydrate in the nature. It is made up of β-(1-4) - linked N-acetylglucosamine (GLcNAc) monomers. Chitin can be found in the exoskeleton of insects, shells of crustaceans and fungal cell wall (Suzuki *et al*., 1998). Annually, more than 80 000 tons of chitin is produced from the marine waste (Patil *et al*., 2000). Chitin is a very stable material, since its function is to protect insects and animals. The ocean contains large amounts of chitin (Pasayat, 2007). Chitin has many application; it can be used in medical materials , agriculture , raw materials and for cosmetics and paper making (Zhang *et al*., 2000; Percot *et al*., 2003). There are three types of chitin: α-chitin involves anti-parallel monomers chain, β-chitin includes parallel monomer chain and γ chitin includes three chains units. α-chitin is the most type found in fungal walls and exoskeleton of invertebrates (Felse and Panda, 2000). Another form of chitin can be found as chitosan which is a modified chitin produced by deacetylation methods of chitin (water –soluble chitin). Chitosans have several properties such as their flexibility which means they can paper in different forms such as fibers, hydrogels, beads, sponges, and membranes (Kandra *et al*., 2012).

5.1.2. Classification of chitinase

Chitin is a complex molecule which is degraded by chitinase. A considerable amount of literature has been published on chitinase classification. Chitinase can be grouped into:

- Endochitinase: these are enzymes which act randomly and degrade internal points throughout the chitin chain.
- Exochitinase: they are enzymes act as the introductory action at the non-reducing ends with release sequential diacetyle chitibiose units (Felse and Panda, 2000).

5.1.3. Chitinolytic microorganisms

Chitinolytic microorganisms are able to degrade chitin by hydrolyzing glycosic bonds. Several bacteria and fungi produce chitinase, including *Serratia marcescens, Trichoderma harzianum* and *Streptomyces spp* (Shaikh and Deshpande, 1993). Many studies have reported that *Bacillus, Aeromonbas, Serratia*, *Vibrio* and some *Pseudomonas* species are the most active chitinolytic bacterial genera. Chitinolytic actinomyces have also been reported, including notably genera of *Streptomyces.* Amongst fungi, *Aspergillus* and *Trichoderma* are the most active chitinolytic genera (Felse and Panda, 2000).

5.1.4 Applications of chitinases

Chitinases can be used for a number of industrial and agricultural purposes. For example in plant pathology, many chitinolytic fungi are used as biocontrol agents against plant pathogenic fungi. For example, chitinase produced by T. *harzianum* were inhibits several harmful fungi (Patil *et al.*, 2000). Several microbial chitinases have been used as antifungal agents such as mixture of chitinase from *S. marcescens* which includes β- glucanase, propan-2-ol and polyoxyethylene lauryl ether; these are sprayed as bio control mixture on a rice field to minimize rice-blight caused by *Pyriculria oryzae*. Under greenhouse conditions, *S. marcescens* has also been used as biocontrol agent against *Sclerotium rolfsii* which attacks beans fields and *Rhizoctonia solani* which attack cotton fields. In addition, chitinase produced by *Arthrobacter sp.* inhibits the growth of *Fusarium moniliforme* which causes pine pitch canker (Shaikh and Deshpande, 1993).

The work described in this Chapter investigated chitinolytic bacteria and fungi and evaluated chitinase production by these isolates as proof of their chitinolytic potential.

5.2. Material and methods

Experiments were conducted to examine the growth of chitinolytic microorganisms on chitin-rich media.

5.2.1. Colloidal chitin preparation

Chitin is a difficult substrate for microorganisms to degrade so it must first be acid hydrolysed to make it more available as a substrate, i.e. colloidal chitin. Colloidal chitin was therefore prepared from crab shell chitin purchased from Sigma. Commercial chitin (10 g) was sieved and then was added to 120 ml of 85% phosphoric acid H_3PO_4 and kept a fridge 4 °C for 24 hours. Tap water was used to remove the jelled-white material which was then

separated by filtration through four layers of cheesecloth, and washed many times in order to remove the acid completely. The colloidal chitin was then stored at 4 °C until use (Rojas-Avelizapa *et al*., 1999).

5.2. 2. Growth medium and culture conditions

Fungi were isolated from an agricultural soil amended with chitosan (Sigma) using the medium (SM) described by Severgnini (2006). After colony purification, the isolates were grown on synthetic medium containing: Colloidal chitin 15g, yeast extract 5g, $(NH_4)_2SO_4$ 1g, $Mg_2SO_4.7H_2O$ 0.3g, KH_2PO_4 1.36g and 20 g agar in 1000 ml deionised water. The pH was adjusted to 5.5. Isolation of fungi was carried out as follows: 25 g of soil were added to a flask containing 250 ml of sterile deionised water. From 10^{-1} to 10^{-5} dilutions, 0.1 ml of each dilution was inoculated on an SM agar medium and incubated at 25°C for 7 days. Colonies on the SM agar plates were purified on the same medium. Developed colonies were then, maintained on potato dextrose agar (PDA).

5.2.3. Qualitative assay for chitinolytic microorganisms

Many microorganisms produce chitinase when they grow on medium containing chitin. For chitin degrading enzyme production, qualitative assays historically have been considered as a powerful tools used for screening bacteria and fungi for chitin-degrading enzyme production.

5.2.3.1. Screening for chitinolytic fungi by plate assay

Different media have been used by different researchers using both physical and chemical criteria for chitin degrading enzyme production.

<u>Screening medium</u>

The medium described by Agrawal and Kotasthane (2009) was used. The chitinase detection medium consisted of a basal medium containing: 4.5 g of colloidal chitin, 0.3 g of $Mg_2SO_4.7H_2O$, 3.0 g of $(NH_4)_2SO_4$, 2.0 g of KH_2PO_4, 1.0 g of citric acid, 30 g of agar, 0.15 g of bromocresol purple and a few drops of Tween-80, in 1L dH_2O). The pH was adjusted to 4.7 and then the medium autoclaved at 121 °C for 15 min. After cooling the medium was poured into plates and allowed to solidify.

<u>Fungal strains</u>

A total of 14 fungal isolates were tested. The fungal isolates were examined using both media: five unknown fungal isolates were obtained from an agricultural soil amended with chitosan, *Mucor hiemalis* (isolated from feather), *Aspergillius flavus, Penicillium*

verrclusium, Motriella amobia, Aspergillius niger (isolated from hair) *Trichophyton mentagrophytes, Aspergillius oryza, Acremonium strictum* and *Pencillium daleae* (obtained from G10 laboratory), 8mm disc of each fungal culture was inoculated on plates containing the test medium, incubated at 25 °C for 1-2 days. The presence of chitinase activity was visualized as a purple zone against yellow background http://www.isth.info/methods/method.php?method_id=11

5.2.3.2. Screening for chitinolytic bacteria by plate assay

Laboratory bacterial samples were tested for chitin production. This test was performed in the same medium used for fungi. *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, Bacillus thuringiensis, Escherichia coli (E.coli), Cupriavidus necator, Bacillus megaterium* and *Rhizobium sp.* All bacterial species were inoculated and the presence of chitin degrading enzyme was then screened qualitatively.

5.2.4. Quantitative assay of chitinolytic fungi

Based on plate's assays, *Mucor hiemalis* and unknown fungi (isolated from soil amended with chitosan) were selected as good chitinase producers. Yeast-nitrogen base (YNB) medium produced from Sigma was used supplemented with chitin. The medium consisted of 6.7% YNB, 0.2% (wt/v) colloidal chitin and 0.5% yeast extract in 1L dH_2O (Watanabe *et al*., 1990). 8mm disc of above samples were inoculated into broth medium and incubated for 3,5and 7 days at 25°C in 150 rpm. The medium was filtered and chitinase activity was measured. Chitinase activity was monitored using the following methods:

5.2.4.1. Nelson method

This method involves the following:

Alkaline copper tartrate regent

- Solution (A): 2.54 g anhydrous sodium carbonate, 2 g sodium bicarbonate, 2.5 g potassium sodium tartrate and 20g anhydrous sodium sulphate were dissolved in 80 ml dH2O then diluted up to 100ml.
- Solution (B): 15g copper sulphate was dissolved in small amount of dH2O. One drop of sulphuric acid was added and the solution diluted upto 100 ml.In order to make alkaline copper tartrate reagent; 4ml of solution B was added to 96 ml of solution A before use.

Arsenomolybdate reagent

2.5 g ammonium molybdate was dissolved in 45 ml dH2O and 2.5 ml sulphuric acid was added and mixed completely. 0.3 g of disodium hydrogen arsenate was dissolved in 25ml

dH2O and mixed. The solution was incubated at 37°C for 24-48 hours (Somogyi, 1952).This assay was aimed at determining glucose release from colloidal chitin as the main reducing sugar (RS) products in the medium (Elyakova, 1972). The reaction mixture contained 500 µl supernatant, 500 µl of substrate 2.0% (w/v) of colloidal chitin in sodium acetate buffer 0.05M pH6.6. The mixture was incubated at 37°C 30min and 500 µl of alkaline copper tartrate regent was added and boiled 10 min, and after cooling 500 µl arsenomolybdate regent was added. The mixture was then diluted with 2 ml of deionized water and the colour was measured at 620 nm. Chitinase activity was then determined by reference to standard curve of D-glucose at 620 nm.

5.2.4.2. Dinitrosalicylic acid methods (DNS)

This assay was used to determine N-acetylglucosamine (GlcNAc) from the colloidal chitin as main reducing sugar (RS) products in the medium. Colorimetric regents described by (Miller, 1959) was used for the estimation of chitinase production. Degradation of chitin was examined using the Rojas-Avelizapa *et al*.,(1999) method with a slight modifications. The reaction mixture contained 100 µl of supernatant, 100 µl of substrate1.0% (w/v) of colloidal chitin in was incubated at 40 °C for 10 min. 400 µl of DNS regent (12.4% sodium hydroxide, 0.63% g DNS, 0.5% phenol, 0.5 % sodium sulphite, and 18.2% Rochelle salt) was added to the mixture and boiled for 15 min. 4.4 ml with deionized water was added and the colour was measured at 535 nm. The chitinase activity was then determined by reference to a standard curve of N-acetylglucosamine (GlcNAc) prepared from a standard solution of N-acetylglucosamine (GlcNAc) (see Appendix A).

5.2.5. Quantitative assay for chitinolytic bacteria

In order to detect the reducing sugars in broth medium, selected bacterial strains **(***Bacillus cereus*) were used depending on the positive results obtained from plate assay. The bacterium were grown into YNB medium (50 ml in 250 ml Erlenmeyer flasks) supplemented with colloidal chitin for 12, 24, and 48 and at 37°C on an orbital shaker (200 rpm), then the chitinase activity was measured using the Nelson method as described above. In addition, pH and cell growth (OD 600 nm) were measured.

5.2.6. Determination of nitrate and ammonium produced in an agricultural soil amended with chitosan

Agricultural soil (50g) was placed in polythene bags and amended with 5g chitosan (Sigma) and mixed thoroughly. The same methods mentioned in previous chapter were used as evolution of macronutrients (ammonium and nitrate).

Statistic analysis of data

Sigma Plot© (Version12.0) was run to analyze the data. $P < 0.05$ was considered as significant. Matching three samples t-test was also performed to check whether there were significantly different.

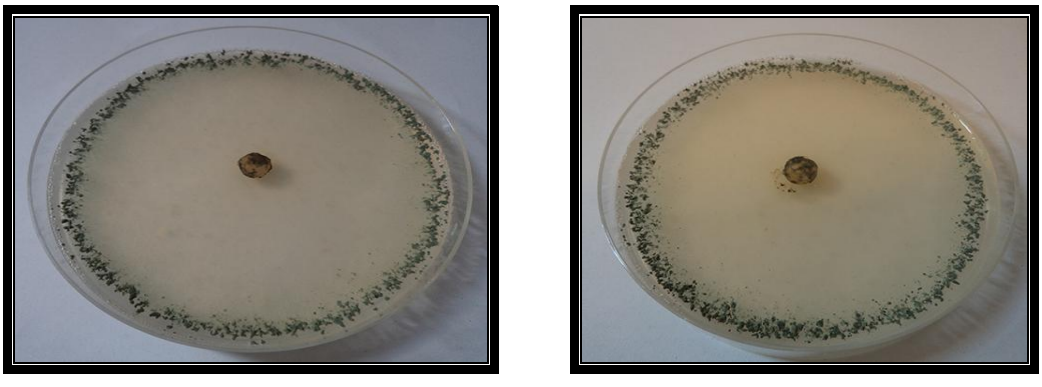
5.2.7. Molecular identification techniques for fungi

Genomic DNA was isolated from five strains (CH3,CH12, CH24,S7 and S12) grown in PDB using a Norgen Fungi Genomic DNA Isolation Kit (GENEFLOW LIMITED, Labstore, UK) by procedures described in chapter 2. The polymerase chain reaction (PCR) amplification of fungal gene was also performed, as described in Chapter 2.

5.3. Results and Discussion

5.3.1. Isolation of chitinolytic fungi

Fungal isolates grew well on medium containing chitosan and colloidal chitin as sources of carbon. Most fungal isolates belonged to *Trichoderma* species (Fig. 5.1), which are known to use a variety of substrates as carbon and nitrogen sources. The needs of carbon and energy of *Trichoderma* can be achieved using monosaccharides, disaccharides and complex polysaccharides (Papavizas, 1985).



Trichoderma harzianum. *Trichoderma tawa.*

Figure 5.1: Fungi isolated from agricultural soil amended with chitosan after 3days incubation in alginate medium.

Colloidal chitin after acid hydrolysis (Fig. 5.2) enhanced fungal growth in the absence of carbon source.

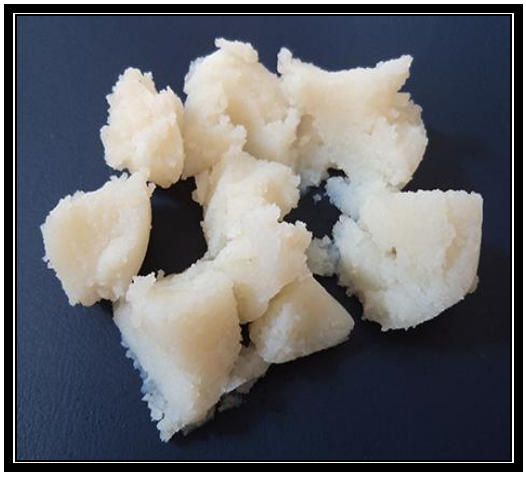
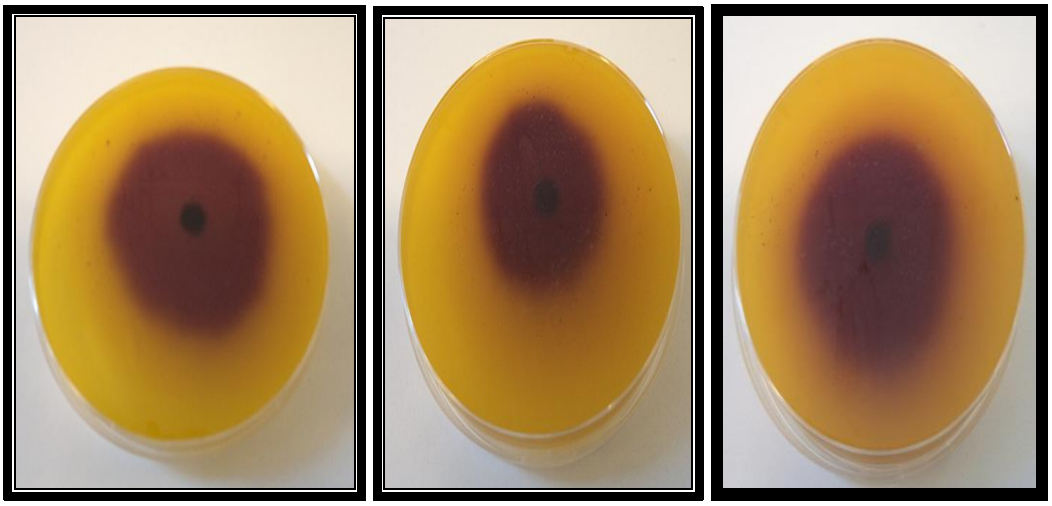


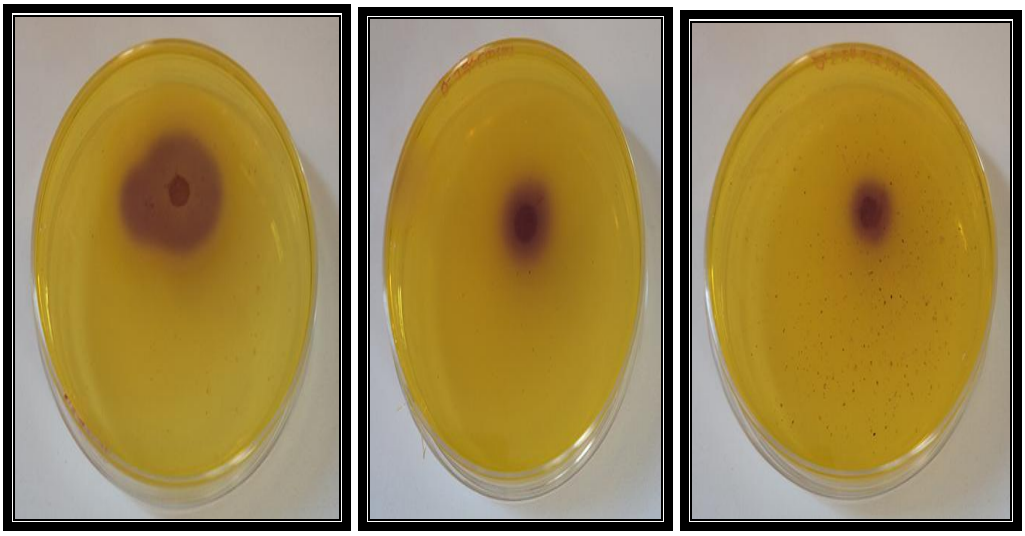
Figure 5.2: Colloidal chitin after acid hydrolysis.

5.3.2. Plate assay for chytinolytic fungi

The objective of plate assay was to ensure that any fungus tested is chitinolytic before any further assessment. Figure 5.3 shows the purple zone as an indicator of chitinase production. Based on bromocresol purple stain (yellow colour at initial pH 4.7) in the test medium; chitinolytic fungi changed the pH of the medium which became alkaline, resulting in a purple colour. All *Trichoderma* species showed chitinolytic activity. Interestingly, *Trichoderma harzianum* and *Penicillium dahleae* showed large purple zones even after short incubation times (Table 5.1). However, *M. hiemalis, P. verruculosum* and *A. strictum* produced only small zone. Chitinase production from *Trichoderma harzianum* and *Penicillium* species has been supported by Binod *et al*., (2005) study. Studies by Binod *et al*., (2005) and Mucha *et al*., (2006) also show that *M. hiemalis* has a high chitinolytic potential.



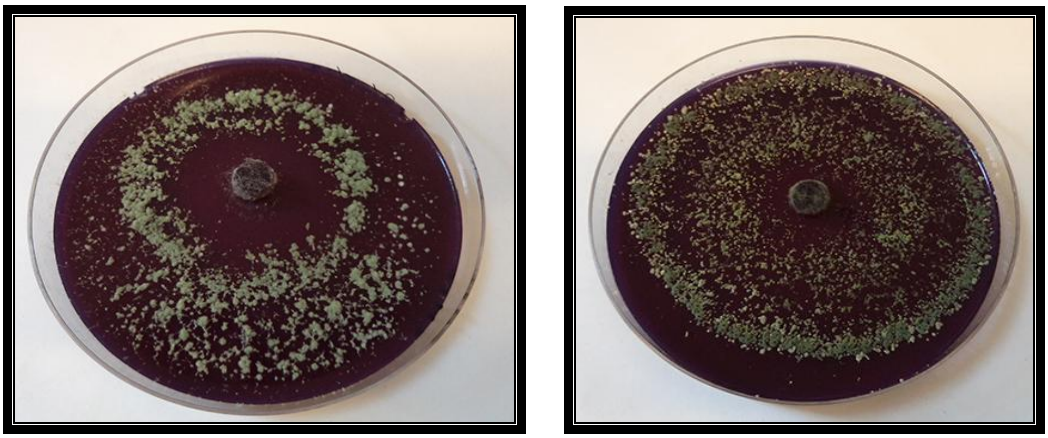
T. harzianum (soil). *T.tawa* (soil). *T.citrinoviride* (soil).



M. hiemalis (feather). *P. Verruculosum* (hair). *A. Strictum* (desert).

Figure 5.3: Comparison of detection of purple zone produced by fungi in test medium after 3 days of incubation.

The results show that marked chitinase production was seen after 6 days of incubation. As a result, the purple zones developed to cover whole area of the plates (Fig. 5.4). Also, the results (Table 5.1) demonstrated the efficiency of the screening method for highly chitinolytic isolates obtained from the soil. The finding shows that the presence of significant difference in their chitinolytic ability between these isolates.



Fusarium sp.(soil) *Trichoderma tawa* (soil)

Figure 5.4: A purple zone produced by fungi in test medium after 6 days of incubation.

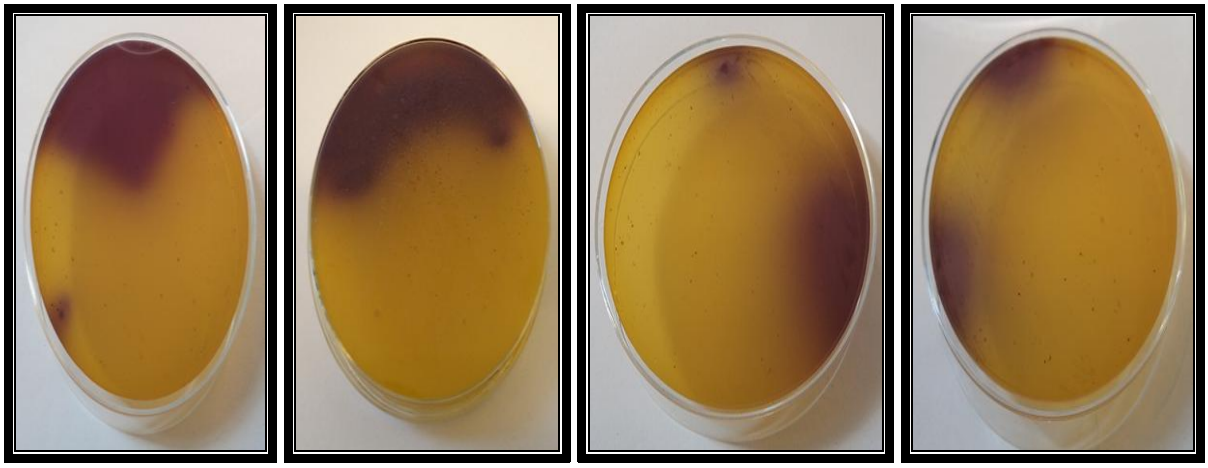
The most striking result to emerge from this study is that hydrolytic activity was recorded against crab shell chitin with varying ability in regard to chitinase production.

Table 5.1: The size of purple zone produced from chitinolytic fungi after 3 days of incubation.

Fungal strain	Chitinolytic activity	Purple zone size (mm)
Trichophyton mentagrophytes (lab)	Negative	–
Penicillium verruculosum (hair)	Positive	17.00 ± 0.57
Penicillium daleae (lab)	Positive	41.66 ± 7.17
Acremonium strictum (desert)	Positive	12.33 ±0.33
Motriella amobia (hair)	Negative	–
Alternaria tenuissima (lab)	Positive	28.33 ±3.17
Fusarium sp.(soil)	Positive	35.66± 1.76
Trichoderma harzianum (soil)	Positive	40.00 ±0.57
Trichodema tawa (soil)	Positive	24.00 ±1.15
Trichoderma citrinoviride (soil)	Positive	28.33± 1.66
Mucor hiemalis (feather)	Positive	33.00±2.08
Aspergillus oryza (lab)	Positive	60.33±5.04
Aspergillus flavus (hair)	Positive	52.33±2.02
Aspergillus niger (hair)	Positive	43.00±1.52

5.3.3. Plate assay for chytinolytic bacteria

The hydrolytic chitinase enzyme was applied to bacterial isolates grown on colloidal chitin as carbon sources. Figure 5.5 shows at range of chitinolytic activity amongst the isolates, i.e. the isolates *P.aeruginosa*, *E.coli*, *C. necator* and *Rhizobium sp* were found to differ in the production of the resultant purple area, an effect which was correlated with their chitinolytic ability.



P.aeruginosa (lab) *E.coli* (lab) *C. necator* (lab) *Rhizobium sp* (lab)

Figure 5.5: The purple zone produced by bacterial strains in test medium after 48 hours of incubation.

The results in Table 5.2 show that chitinase production was not observed in *Staph. aureus* and *B. thuringiensis;* moreover, these isolates were not able to grow at low pH.

Table 5.2: Chitinolytic activity in plates produced from bacteria after 48 hours of incubation.

Bacterial strains	**Chitinolytic activity**
Pseudomonas aeruginosa	Positive
Bacillus cereus	Positive
Staphylococcus aureus	Negative
Bacillus thuringiensis	Negative
E.coli	Positive
Cupriavidus necator	Positive
Bacillus megaterium	Positive
Rhizobium sp.	Positive

5.3.4. Qualitative analysis of chitinolytic fungi

The ability of fungi to use colloidal chitin as a carbon source was tested in order to identify fungal chitinase producers. Chitinase was assayed by the release monomers of N-acetyglucosamine (GLcNAc) as end products of chitin breakdown. The activity is expressed

as reducing sugars (GLcNAc). *Trichoderma harzianum* and *Mucor hiemalis* were selected to evaluate the chitinase production and to measure the amount of GLcNAc. Figure 5.6 shows the chitinolytic enzyme secreted by *Trichoderma harzianum* into the culture medium and the products of chitin hydrolysis. When these fungi were grown on MS medium plus colloidal chitin as carbon source, after 3 days the GLcNAc was observed. The GLcNAc products reached the maximum value by *T. harzianum* after 5 days. The enzyme then decreased sharply after 7 days. The reason might be due to accumulation of the products of chitin breakdown as shown by Pinto *et al*., (1997).

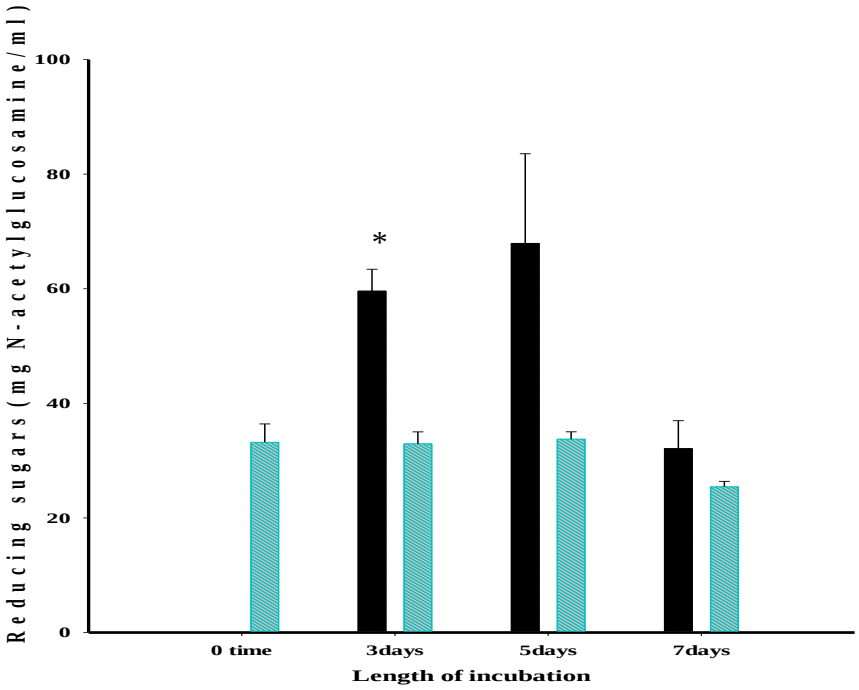


Figure 5.6: Monomers of N-acetyglucosamine produced from *T. harzianum* during 7 days incubation. *T. harzianum* (▬) and control (▨). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

High levels of chitinase during the early incubation period lead to the degradation of chitin and release of monomers. The finding provides evidence that *T. harzianum* is a chitinolytic fungus. It can clearly see that *T. harzianum* showed high activity against chitin and released GLcNAc in the medium. El-Katatny *et al.*, (2001) found that *T. harzianum* has high activity towards chitin, which is in good agreement with the results of the present study. Also, Elad *et al.*, (1982) found that *T. harzianum* released chitinase when grown on chitin as carbon source. Ulhoa and Peberdy, (1991) also found that significant levels of chitinase produced from *T. harzianum* mycelium when the fungus was incubated with chitin in the absence of

any other compounds. Similarly, chitinase produced from *Mucor hiemalis* released GLcNAc from colloidal chitin. The level of lytic enzyme released from *M. hiemalis* shown in Figure 5.7.The highest activity was seen after 5days incubation then this value decreased. Mucha *et al*., (2006) reported that chitinase activity was similarly seen in *M. hiemalis.* Interestingly, as with *T. harzianum*, the highest chitinase production was recorded after 5days. Chitinase secretion in the test medium is promoted by chitin (Elad *et al*., 1982).

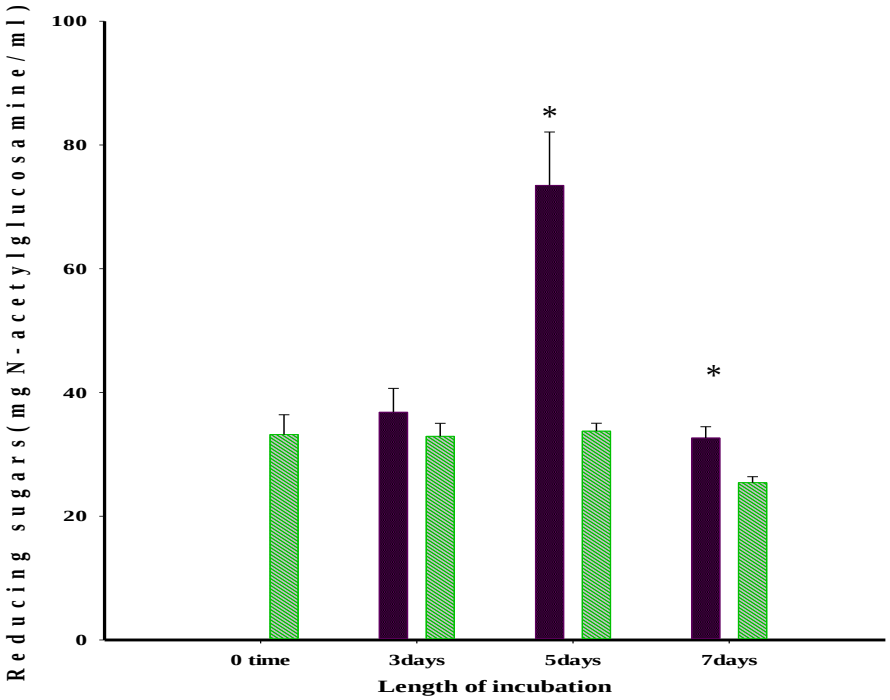


Figure 5.7: Monomers of N-acetyglucosamine produced from *M. hiemalis* during 7 days incubation. *M. hiemalis* (▬), and control (▬). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

Figure 5.8 shows the pH values produced by *M. hiemalis* and *T.harzianum.* Based on these results, the highest pH value was found to be 6.5 produced by *T.harzianum* and 6.3 by *M. hiemalis* after 7 days incubation. This trend is the opposite of chitinase production, so there is no obvious correlation between GLcNAc residues and pH. A significant difference was detected in pH rate between the both fungi and control, due to alkalinization of the medium and the ability of the fungus to release ammonia.

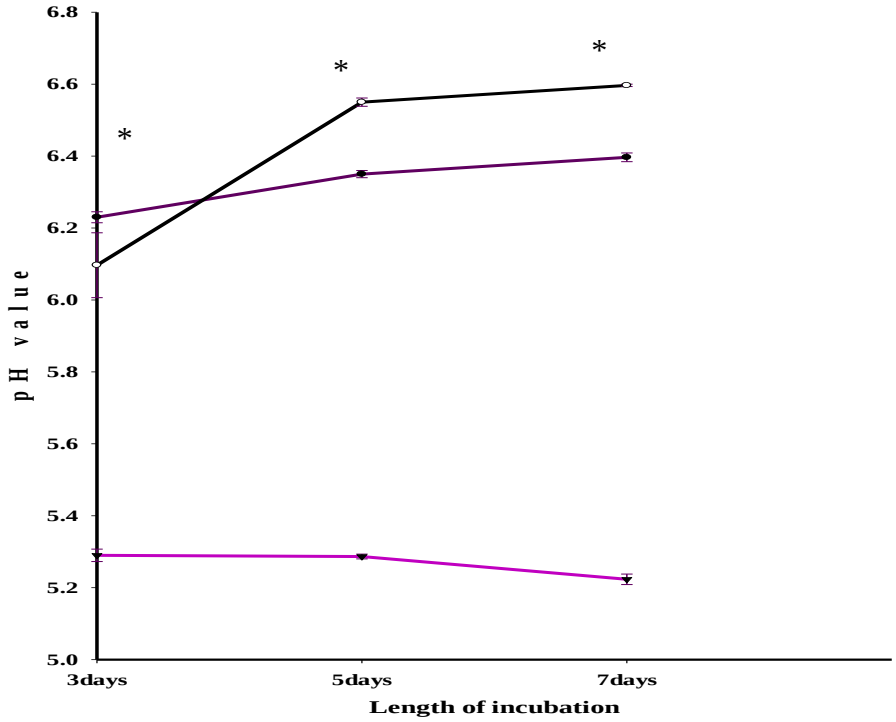


Figure 5.8: pH from *T.harzianum* and *M. hiemalis* in test medium. *M. hiemalis* (—●—),*T.harzianum* (—○—) and control (—▼—). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

Biomass production during the hydrolysis of chitin by *T.harzianum* and *M. hiemalis* after 7days was also determined (Fig. 5.9).

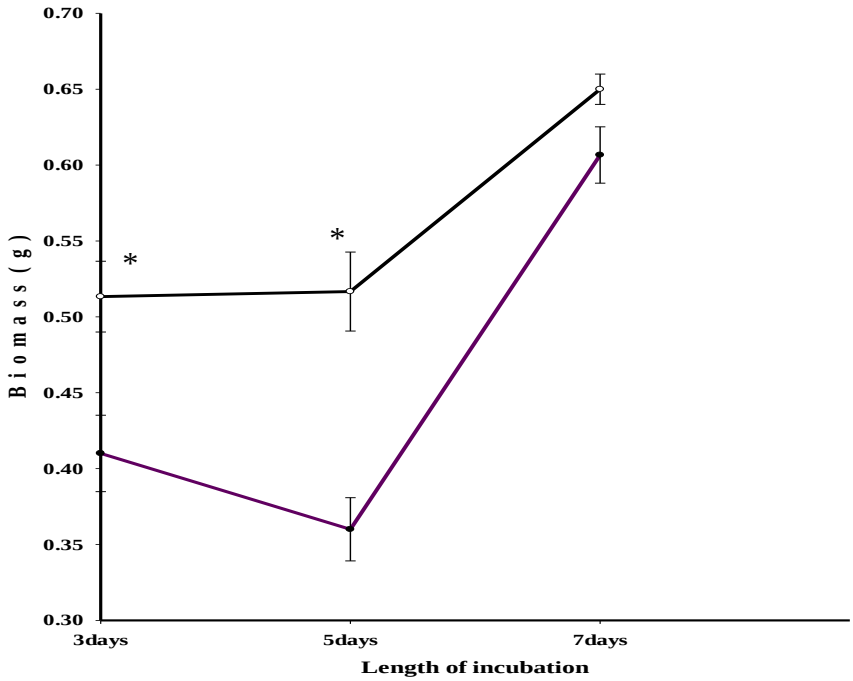


Figure 5.9: Biomass production from *T.harzianum* and *M. hiemalis. M. hiemalis* (—●—) and *T.harzianum* (—○—). Means of triplicates (±) standard error. *significant difference ($P < 0.05$).

Good growth was supported by reducing sugars and there was a gradual decrease at 5 days in the fungal growth. The highest value was reached at 7 days of incubation in both fungi. It is noticeable that there was no connection between the enzyme production and biomass.

5.3.5. Qualitative analysis of chitinolytic bacteria

Chitinase in bacteria was examined also by release of free glucose as end products from chitin. The activity was expressed as reducing sugars (glucose). The production of chitinase by *B.cereus* is shown in Figure 5.10. Chitinase activity is seen to peak after 24 hours incubation in YNB medium plus chitin. Activity then decreased after 48 hours. *B.cereus* degraded colloidal chitin efficiently and produced free glucose. Based on these results, this strain exhibited high chitinolytic activity. This result confirmed that colloidal chitin was used as a sole carbon source for chitinase production and the release free glucose groups by *B.cereus.* Several other studies confirm that *B.cereus* releases chitinase when colloidal chitin is used as carbon source (Chang *et al.*, 2003 ; Wang *et al.*, 2001; Wang *et al*., 2009).

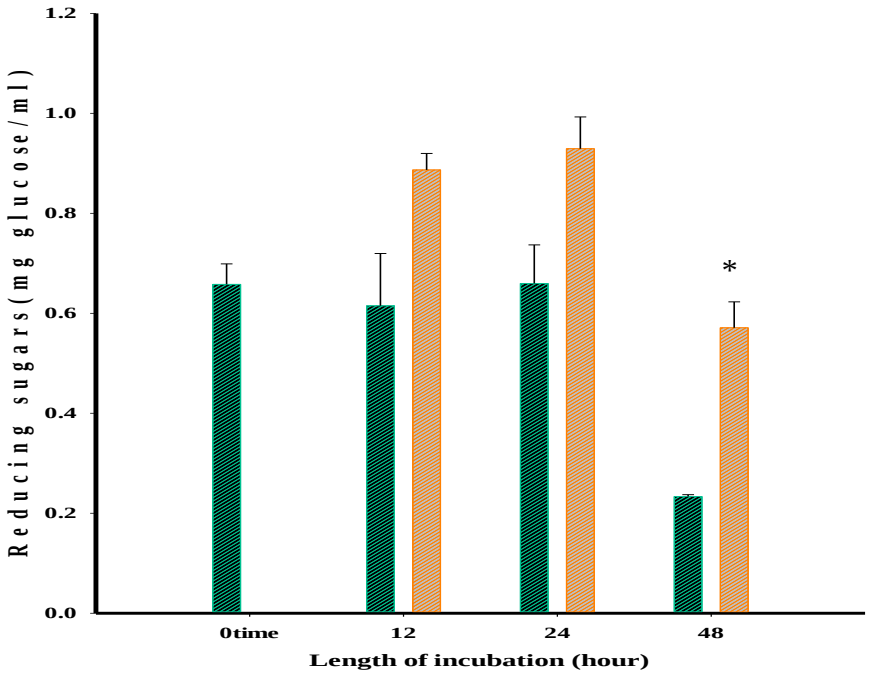


Figure 5.10: Free glucose produced from *B.cereus* after 48 hours of incubation. *B. cereus* ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

The pH of the medium was measured in order to determine any the correlation between chitinase activity and pH. Under the conditions used, pH reached a maximum value after 24 hours incubation at 7.6 (Fig. 5.11). The maximum production of chitinase was also detected after 24 hours, showing a possible relationship with medium pH.

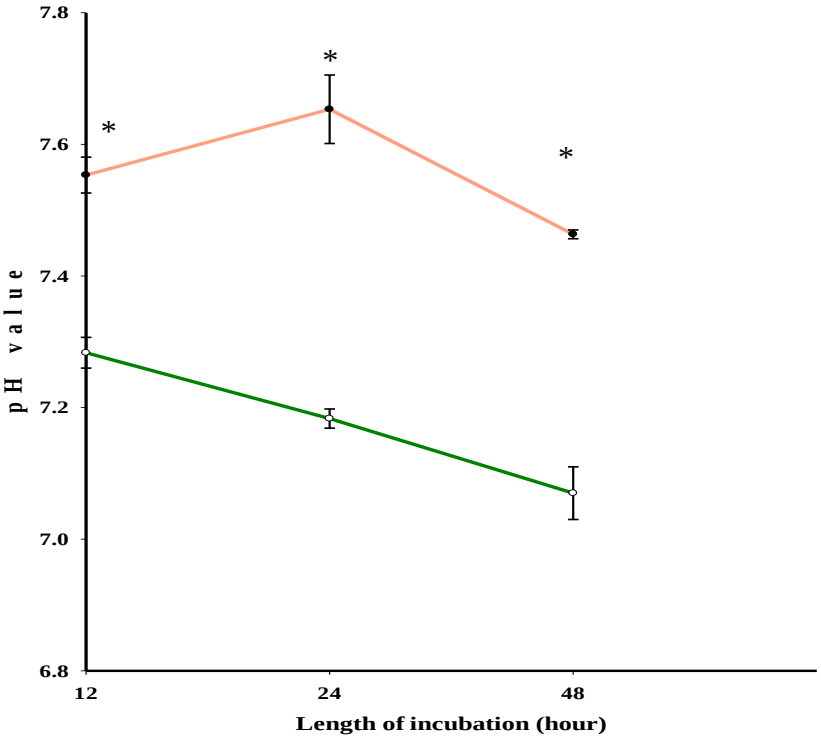


Figure 5.11: pH value produced from *B.cereus* after 48 hours of incubation. *B. cereus* ( ) and control ( ). Means of triplicates (±) standard error. *significant difference from the control ($P < 0.05$).

Figure 5.12 shows that optimal growth of *B.cereus* on YNB medium supplemented with colloidal chitin. *Bacillus cereus* grew on chitin as sole carbon source with growth increasing gradually after 24 hours of incubation and a sharp increase in the growth after 48 hours incubation.

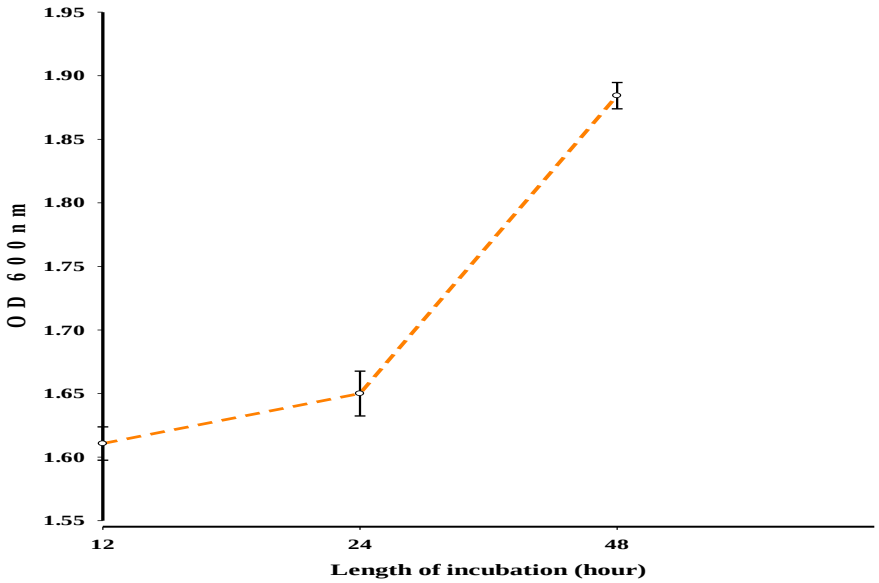


Figure 5.12: Growth rate of *B.cereus* in YNB medium supplemented with colloidal chitin after 48 hours of incubation. Means of triplicates (±) standard error.

It can be clearly seen that the maximum growth occurred after 48 hours and the lowest growth was after 12 hours.

5.3.6. Chemical analysis of agricultural soil amended with chitosan

Here, chitosan was used as nitrogen source instead of $(NH4)_2SO_4$ and urea.

5.3.6.1. Nitrate production

Nitrate levels in agricultural soil amended with chitosan are shown in Figure 5.13.The amount of nitrate increased gradually and peaked after 4 weeks. Nitrate production occurred over the entire incubation period, this might be due to the chitin containing amino sugars and the microorganisms produced lytic enzymes for the decomposition of chitinous material for use as carbon and nitrogen sources. In addition, the activity of the extracellular enzymes seems to be associated with the rate of decomposition and mineralization of chitosan. It can be clearly seen that the microorganisms in the soil used here attacked the chitosan and produced enzymes to obtain energy and nutrients.

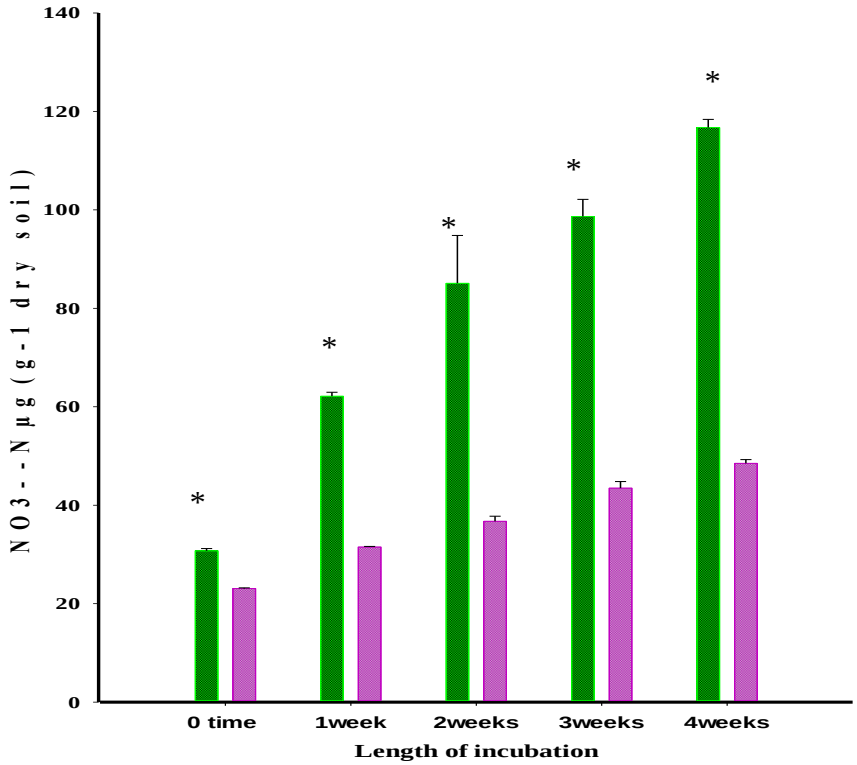


Figure 5.13: Nitrate production from the hydrolysis of chitosan. Treatment (**)** and control ( ). Means of triplicates (±) standard error**.** *significant difference from the control ($P < 0.05$).

5.3.6.2. Ammonium production

Figure 5.14 shows the concentration of ammonium produced from the decomposition of chitosan. The main observation is that the highest rate of ammonium was seen at week one, while only a small production of ammonium occurred over the following weeks. This might be because ammonium accumulation prevented further degradation of chitosan. In contrast, there was no change in control. The oxidation of ammonium from the hydrolysis of chitosan was also found to be high at 1week only compare to following weeks. The lower ammonium production might be due to accumulation of carbon dioxide and ammonia which inhibited the synthesis of microbial enzymes.

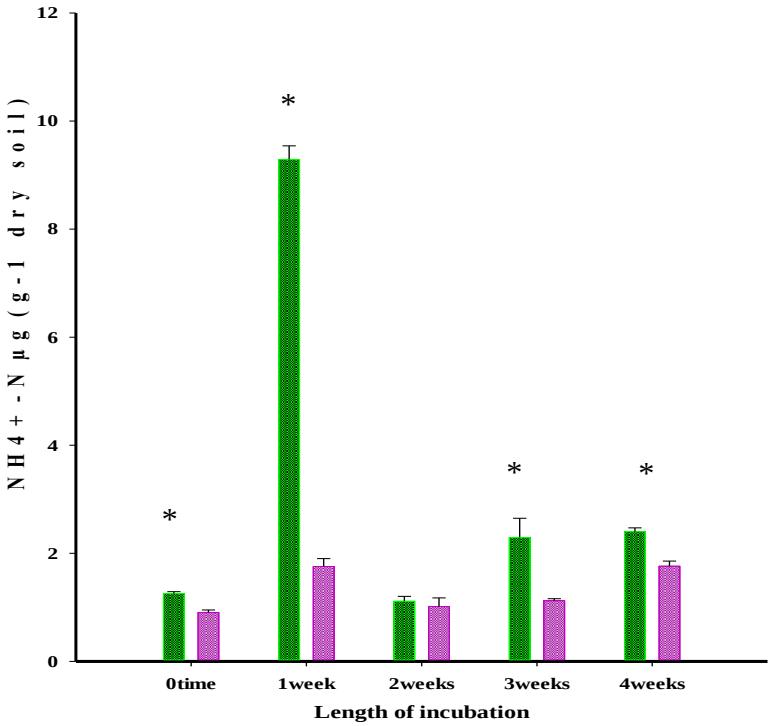


Figure 5.14: Ammonium production from the hydrolysis of chitosan. Treatment ( ) and control ( ). Means of triplicates (±) standard error. *****significant difference from the control ($P < 0.05$).

5.3.7. Phylogenetic identification of unknown fungi

Four strains of unknown fungi were isolated from agricultural soil amended with chitosan and designed as CH3, CH12, CH24 and S7. The first step in the identification of unknown fungal gene was the amplification of genomic DNA using ITS1 and ITS4 primers. The fragments of PCR products observed in the lanes shown in Figure 5.15.

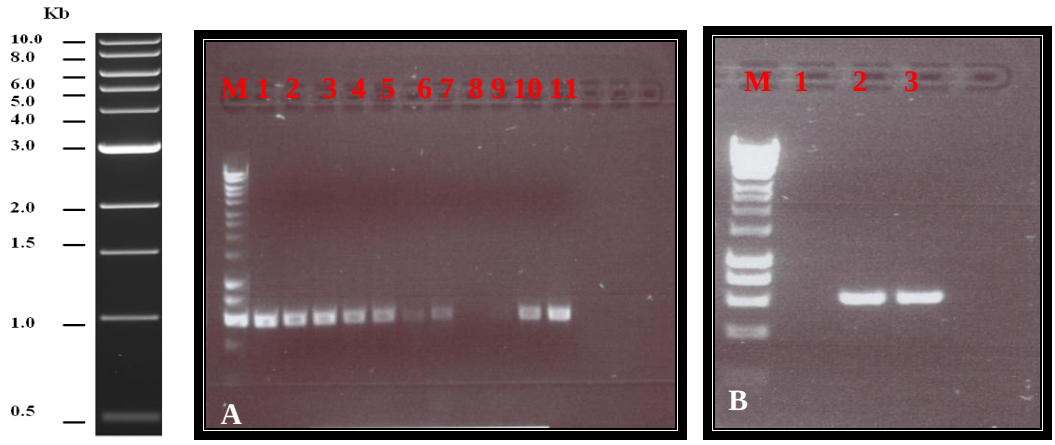


Figure 5.15: Amplification of fungal genomic DNA from unknown isolates. (A) Lane 10: *Trichoderma harzianum (*B) Lane 2: *Trichoderma tawa* and Lane 3: *Trichoderma citrinoviride.*

The fungal gene sequence identity is presented in Table 5.3. The sequence from fungal isolates was obtained and compared with the databases.

Table 5.3: Fungal sequence analysis of isolated fungi.

Source of Sample	Representative sequence	Closest matches identification	Sequence identity	Length of sequence (bp)	NBCI (Accession number)
Soil amended with chitosan	S7	*Trichoderma citrinoviride*	99%	621	HQ596929.1
	CH3	*Trichoderma harzianum*	100%	549	JX982444.1
	CH24	*Trichoderma tawa*	99%	613	KC847172.1

The single most striking observation to emerge from the data comparison was *Trichoderma harzianum* showed high identity 100%. Similarly, *Trichoderma citrinoviride* and *Trichoderma tawa* showed 99% identity. Blast analysis of CH3 showed similarity of 100% with *Trichoderma harzianum* (Fig.5.16).

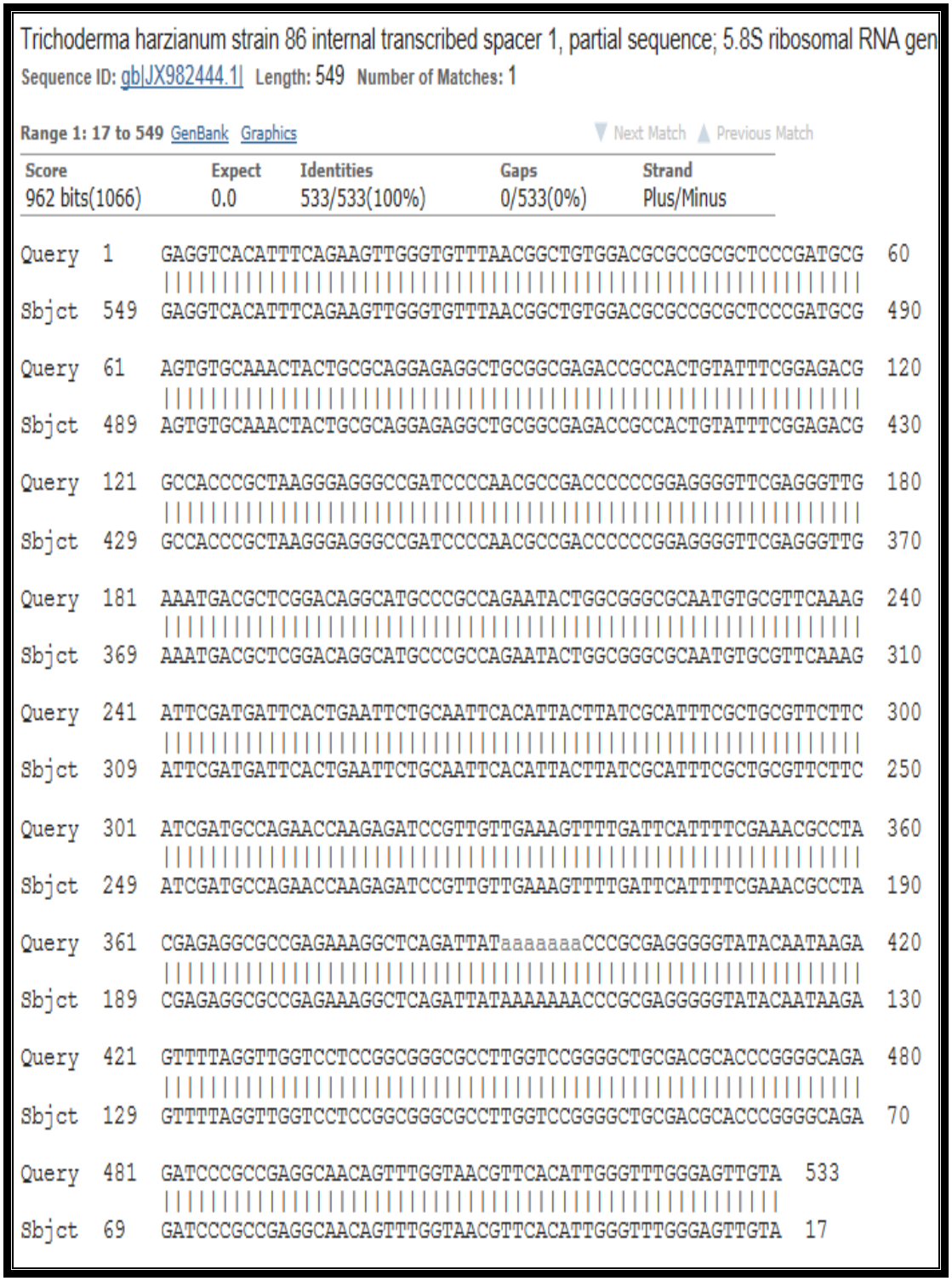


Figure 5.16: Sequence of *T. harzianum* (achieved after the amplification of whole cell genome) referring to the affiliations through BLAST studies .The ‹‹Query ‹‹ referring to the line when input sequence. The subject refers to sequence of line matching.

The fungal isolate was identified (Fig. 5.17) as *Trichoderma harzianum* which is an ascomycete.

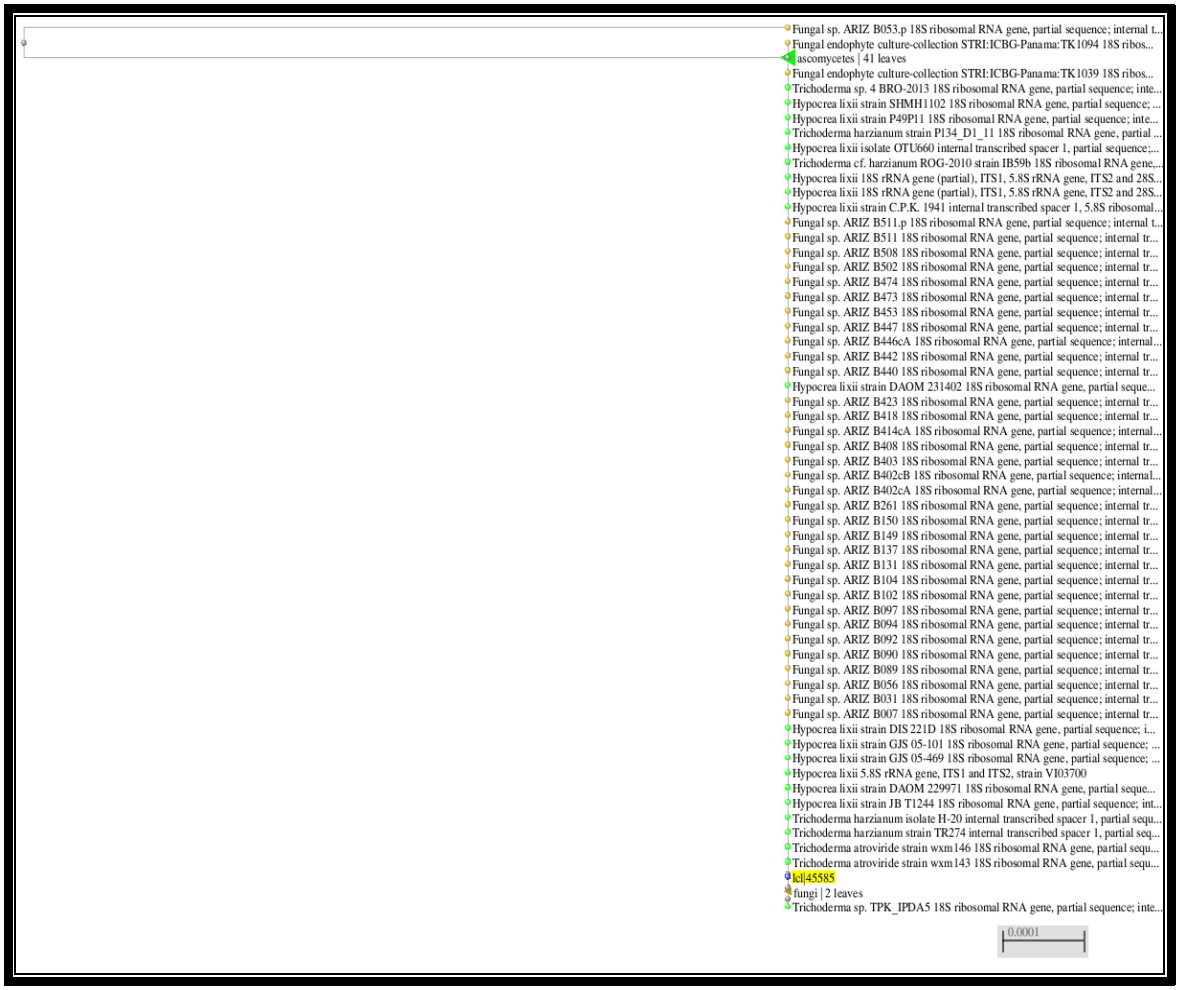


Figure 5.17: Phylogenetic tree of CH3 (*Trichoderma harzianum*).

CHAPTER 6

CHAPTER 6
ISOLATION AND IDENTIFICATION OF CERTAIN ENZYMES

6.1. Introduction

The past thirty years have seen interestingly rapid advances in the field of protein identification. There have been several studies in the literature reporting the difficulty and the challenges of protein identifications.

6.1.1. Using Ammonium sulphate in protein isolation

For protein isolation and purification, several salts such as ammonium sulphate have been used. Ammonium sulphate has many useful properties e.g. it has high solubility and it is inexpensive. Consequently, it has become the most chemical used in protein isolation and purification. Ammonium sulphate fractionation is initial step used in lyase purification from a complex protein mixture (Wong *et al*., 2000).

6.1.1.1. Using ammonium sulphate to isolate alginate lyase

Large numbers of enzymes have been partially purified using $(NH_4)_2SO_4$. Among these enzymes include alginate lyses which have been purified partially from several bacteria species. For example, ammonium sulphate has been successfully used for isolation and partial purification of alginate lyase (crude alginase) from *Flavobacterium sp*. using two saturation values ,30% and 55% (An *et al*., 2008). As highlighted by Baron *et al*., (1994), 50-65% is another saturation value of $(NH_4)_2SO_4$ which can be used to isolate alginate lyase from *Klebsiella peneumonia.* The precipitation of alginate lyase from *Streptomyces sp.* and *Pseudomonas sp* has also been achieved using ammonium sulphate followed by the SDS-PAGE to confirm the purity of the purified lyase (Cao *et al*., 2007; Muramatsu and Sogi, 1990).

6.1.1.2. Using ammonium sulphate to isolate keratinase

Ammonium sulphate with a saturation of 80% has been utilized with the supernatant of *Streptomyces sp.* in order to purify keratinase then the purity was checked using SDS-PAGE gel (Xie *et al*., 2010). Also, 80% saturation of ammonium sulphate has been used as first step of keratinase purification from the supernatant of *Trichophyton vanbreuseghemii* (Moallaei *et al*., 2006). Cheng *et al*. (1995) also reported that the culture supernatant of *Bacillus licheniformis* can be precipitated using ammonium sulphate 80% followed by dissolving in suitable buffer.

6.1.1.3. Using ammonium sulphate to isolate pectinase

Microbial pectinase has been isolated with ammonium sulphate efficiently. A study reported that Exo-PG, Endo-PG and pectinesterase of *Trichoderma reesi* have been salted out successfully using ammonium sulphate (Gummadi and Panda, 2003). In fact, several types of pectinase have been prepared from culture of fungi, bacteria and yeast using ammonium sulphate. As a result, ammonium sulphate fractionation is one of the common methods to purify pectinase lyase near to homogeneity (Yadav *et al*., 2009). Generally, ammonium sulphate can be used for crude enzyme preparation such as crude endo-polygalacturonase from *Penicillium sp*. precipitated using ammonium sulphate with two saturation from 30% to 80% (Yuan *et al*., 2011).

6.1.1.4. Using ammonium sulphate to isolate chitinase

For the preparation of crude chitinase enzyme from *Bacillus circulans,* ammonium sulphate has been used with concentration 80% then the precipitate protein dissolved in the buffer (Watanabe *et al*., 1990). In a different study, Suzuki *et al*., (1998) found that 80% saturation of ammonium sulphate can be used to isolate chitinase from the supernatant of *serratia marcescens.* A partially purified chitinase can be produced using 100% $(NH_4)_2SO_4$ from the filtrate of *Bacillus cereus* as reported by (Kishore and Pande, 2007). Chitinase isolation at 40-70% saturation has also been reported (Huang and Chen, 2004), as has a 60.8% saturation using *Bacillus cereus* supernatant (Wang *et al*., 2009).

6.1.2. Identification of proteins using mass spectrometry data (MS)

Mass spectrometry is one of the most commonly used methods for the identification of proteins. Many computer programs have been characterized for proteins identification by searching a sequence database using mass spectrometry data (Cottrell and London, 1999). Mass spectrometry techniques are powerful tools in protein identification and they are widely used. For example, samples containing just a few protein components can be applied using MS. Also, complex samples containing many proteins, such as clinical samples, can also be identified. In addition, mass spectrometry can be used without previous knowledge of the proteins to be analyzed and identified (Edwards, 2011). There are several approaches for mass spectrometry:

- **MS/MS ion searches** which use MS/MS data derived from one or more peptides.

- **Peptide mass fingerprinting** which uses peptide molecular weight produced from protein digestion by the enzyme.

- **Sequence query** which combine the mass data from MS with amino acid sequence data or physicochemical data that deduce sequence or composition (Cottrell and London, 1999). The general approach is that the protein sample is digested to produce short peptides via proteolytic enzyme then the peptides will separate based on physical and chemical properties of peptides. Because most peptides have similar physicochemical properties the MS can achieve a scan in order to choose the most abundant peptide ions for analysis. A selected peptide ion is known as the tandem mass-spectrum or MS/MS spectrum. There is type of computer software called tandem mass-spectrometry search which can be used to identify the proteins by the analysis of datasets. These searches match the MS/MS with peptide sequences produced from a protein sequence database; they then use the identified peptides to recognize the protein in the sample (Edwards, 2011). According to McDonald *et al.*, (2002), liquid chromatography mass spectrometry (LC-MS/MS) can be used to analyze several peptides derived from digested bands determined via polyacrylamide gel electrophoresis. In fact, LC-MS/MS has been used to analyze a massive number of proteins and it can compare the data from the tandem mass spectrometry to the database sequence and identify the peptides via their amino acid sequence. The aim of the work reported in this Chapter was to isolate four degradative extracellular enzymes from bacteria using ammonium sulphate, namely: keratinase, alginate lyase, pectinase and chitinase, in order to determine their molecular weight. Identification of three enzymes (keratinase, pectinase and chitinase) was also determined using Liquid chromatography mass spectrometry (LC-MS/MS).

6.2. Materials and Methods

Protein purification requires isolation of whole protein from initial materials as a crucial step.

6.2.1. Isolation of alginate lyase as crude alginase from *Pseudomonas aeruginosa* strain ATCC 27853

- **Preparation of the crude extract**

For the first step in the partial purification of *Pseudomonas aeruginosa* ATCC 27853 proteins in the crude state a protein fraction from the cell was obtained. The experiment steps were conducted at 4ºC in order to minimize the possibility of protein degradation. The bacterium was grown in (broth) medium (50 ml in 250 ml Erlenmeyer flasks) containing 1 g

sodium alginate, 1g yeast extract, 2 g tryptone, 5 g sodium chloride in 1000 ml distilled water at pH 7.6. The medium was incubated at 37°C in 200 rpm for 24 hours. The initial step consisted of achieving homogeneity of supernatant to remove cellular debris using a centrifugation at 5 000 x g for 20 min. The pellet was then discarded and the supernatant was applied to the following steps.

- **Precipitation of alginate lyase using two saturations of ammonium sulphate**

During the precipitation of protein, a supernatant was used from a 24 hours culture grown on alginate medium and initially precipitated using ammonium sulphate 30% and held at 4 °C for 2 hours. This supernatant was then centrifuged at 10 000 × g at 4 °C for 15 min, and the pellet was harvested. Ammonium sulphate was added again as additional saturation into the supernatant to give 55%, and then the supernatant was kept at 4 °C for 4 hours. The pellet was collected again by centrifugation at 10 000 × g at 4 °C for 15 min. The obtained pellet was transferred and dissolved in 0.02 mol/L phosphate buffer (pH 7.0). The solution was incubated overnight at 4 °C. The mixture was then centrifuged for 15 min at 5,000 rpm to remove the pellet. The dialyzed supernatant was then used as the crude alginase for the following tests (An *et al*., 2008).

6.2.1.1. Determination the molecular weight of alginate lyase using SDS-PAGE

For this purpose, SDS polyacrylamide gel electrophoresis (SDS-PAGE) was used as follows:

SDS-PAGE

Table 6.1: Buffers stain and de-stain composition.

SDS-PAGE lower buffer	1.5 M Tris/HCl, 0.4% (w/v) SDS, pH 8.8.
SDS-PAGE upper buffer	0.5 M Tris/HCl, 0.4% (w/v) SDS, pH 6.8.
SDS-PAGE running buffer	25 mM Tris/HCl, 0.1% (w/v) SDS, 0.19 glycine pH 8.3.
SDS-PAGE loading buffer	315 mM Tris/HCl, 25% (v/v) SDS, pH 6.8 glycerol, 5% (v/v) β-mercaptoethanol; 7.5 mM bromophenol blue, 18% (w/v) SD
Stain	1 g Coomassie brilliant blue R-250, 40% (v/v) methanol, 10% (v/v) glacial acetic acid, 50% deionised water.
De-stain	30% (v/v) methanol, 10% (v/v) glacial acetic acid, 60% deionised water.

Gel Composition **Table 6.2:** Gel composition.

15% Separating Gel	1.25 ml lower buffer 2.5 ml of 40% (w/v) acrylamide 1.25 ml distilled water 25 µl 10% (w/v) APS (ammonium persulfate) 7.5 µl TEMED
Stacking Gel	6.25 µl upper buffer 300 µl of 40% (w/v) acrylamide 1.575 ml distilled water 25 µl 10% (w/v) APS (ammonium per sulfate) 7.5 µl TEMED

6.2.1.2. Standard Protein marker Mixture

The protein marker used was- Precision plus protein ^{TM} All Blue, produced by Bio-Rad. It is a mixture of 10 blue-stained recombinant proteins arranged from 10 to 250kDa.

6.2.1.3. SDS-PAGE gel electrophoresis

A Bio-Rad mini-protean II apparatus was used to perform SDS-PAGE. The experiment was carried out using two types of gels; polyacrylamide separating gel 15 % and polyacrylamide stacking gel 4 %. Protein sample (alginate lyase) was subjected for loading steps using 5 x SDS-PAGE loading buffer 1:1 ratio. After a period of 10 minutes heating at 90 °C, 20 µl of the sample was loaded into the gel. The apparatus was run at a constant voltage of 100 – 150 V for approximately 75 – 90 minutes. Gels were then stained at room temperature for a maximum of 16 hours. Then they were de-stained by shaking at room temperature until the bands were clearly visible.

6.2.2. Isolation of pectinase, keratinase and chitinase using ammonium sulphate

A variety of methods can be used to precipitate of proteins such as using changes in temperature, pH, add salts and organic solvents. To precipitate the proteins, ammonium sulphate as a classical method was used.

6.2.2.1. Isolation and partial purification of pectinase from *B.megaterium*

Bacillus megaterium was chosen based on its activity against pectin in-vitro. For the extraction of pectinase, *B.megaterium* was grown on YEP broth at 37 °C using 200 rpm for12 hours. Culture supernatant was collected from 12 hours-old culture via centrifugation at 5 000 x g for 20 min. The supernatant was precipitated with 80% saturation ammonium sulphate using the same steps described above. The precipitated protein was then dissolved in Tris-HCl buffer (0.01 M, pH 7.5) and dialyzed in the same buffer overnight (Kashyap *et al.*, 2000).The dialyzed protein obtained was treated as the partially purified enzyme.

6.2.2.2. Isolation and purification of keratinase from *Bacillus licheniformis* strain LZBL-11

Bacillus licheniformis was isolated from marine sand and selected for this test. The selection was based on its activity against keratin. Generally, the bacterium was grown on LB medium supplemented with azure at 37°C using 200 rpm for 24 hours. The supernatant which collected from the 24 hours-old culture centrifuged at 5,000g for 20 min. The resultant supernatant was precipitated with 80% saturation ammonium sulphate using a previously

described method. The precipitated protein was dissolved in Tris-HCl buffer (0.01 M, pH 7.5) and placed in the same buffer overnight (Cheng *et al.*, 1995). The keratinase was expressed as partially purified keratinase.

6.2.2.3. Isolation of chitinase from *B.cereus* strain BS2

Bacillus cereus strain BS2, a bacterium used in this approach was previously assessed as good chitinase producer in chitin medium. The strain was grown at 37°C for 24 hours in YNB medium containing 0.2% chitin and 0.5% yeast extract. All partial purification procedures were done in cold room at 4°C. For enzyme precipitation the following steps were performed.

- **Step1**: **preparation of crude extract**

To obtain a clear supernatant, cell debris was removed by centrifugation at 5000×g for 20min. The supernatant fluid was then taken to the following step.

- **Step2: $(NH4)_2S0_4$ precipitation**

A saturation of 30% $(NH4)_2S0_4$ was added to the supernatant solution. The resultant precipitate was removed by centrifugation at 10000×g for 15min and discarded. Further (NH4)2SO4 was added to obtain 80% saturation. The suspension was again centrifuged at 10000×g for 15min.and then dissolved in 0.02 M sodium phosphate buffer (pH 6.0) and dialyzed overnight against the same buffer (Watanabe *et al*., 1990).

6.2.3. Measurement the activity of proteins

Enzymes are known to be proteins having different solubility in salt solution and procedures for enzyme extraction mostly begin with salt precipitation (commonly ammonium sulphate). When the salt is added serially, individual proteins precipitate according to their solubility. To evaluate the purification step, each step of the extraction must be checked for enzyme activity by measuring the decrease of substrate or the increase in the products. The activity four enzymes were measured using the same methods described in each of the above Chapters (Table 6.3).

Table 6.3: Measurement of the activity of enzymes.

Enzyme	**Activity methods**
keratinase	At 595 nm.
pectinase	DNS at 530nm.
chitinase	DNS at 535nm.

6.2.4. Measurement of the concentration of protein (Bio-Rad assay)

Approximately 0.8 ml of sterilised milliQ water was transferred into a plastic cuvette and 0.2 ml of Bio-Rad dye regent was added to each cuvette tubes. The cuvettes were then covered with parafilm and mixed carefully by centrifugation or tipping upside down and the right way up again. 5µl of protein solution was added and measured at OD 595 using Bio-Rad regent (Bradford, 1976). The protein concentration was calculated as following:

Protein concentration (mg ml^{-1}) = OD_{595}×15/Volume of protein (µl)

6.2.5. Gel electrophoresis (Nu-PAGE® Bis-Tris gel)

There are many types of SDS-PAGE can be used in protein electrophoresis. One of them is Nu- PAGE model. Nu- PAGE conditions were different to previous SDS-PAGE. Nu-PAGE® Bis-Tris gel is precast polyacrylamide gel which designed to give optimal separation of small to medium size of proteins during gel electrophoresis. It has a natural pH that minimizes protein modifications resulting in sharp bands. In addition, it can be used for sequence, mass spectrometry, and any application in which protein integrity is vital. Moreover, NuPAGE® Bis-Tris gel designed to allow separation of a wide range of molecular weight proteins and fast run time (completed in as little as 35 min). Analysis of keratinase, pectinase and chitinase obtained during preparation of protein was carried out using a NuPAGE® Novex Bis- tris 4-12% gel produced by Life technologies. It was run with MES running buffer **(**20X). The buffer consisted of 1X: 50 mM MES, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, and pH 7.3.

Standard Protein marker Mixture and electrophoresis conditions

The marker used is Mark 12 ™ unstained standard (2.5-200 kDa) produced from Life Technologies. The protein marker consisted of : Myosin(200 kDa), B-Galactosidase(116.3 kDa), Phosphorylase b(97.4 kDa), BSA(66.3 kDa), Glutamic dehydrase(55.4 kDa), Lactate dehydrogenase(36.5 kDa), Carbonic anhydrase(31 kDa), Trypsin inhibitor(21.5 kDa), Lysozyme(14.4 kDa), Aprotinin(6 kDa), Insulin B chain(3.5 kDa) and Insulin A chin(2.5 kDa). Proteins samples (chitinase, keratinase and pectinase) were normally prepared with loading buffer. After a period of 10 minutes heating at 90 °C, 20 µl of the sample was loaded onto the gel using voltage of 200 for approximately 35min. The gel was stained with Instant Blue ™ stain (Expedeon) at room temperature. The gels were de-stained in destain solution overnight using shaking at room temperature until protein bands were clearly visible.

6.2.6. Identification of the proteins using mass spectrometric analysis (SM)

The aim of the experiment is to identify component proteins of key bands of interest on SDS-PAGE pertaining to the clients derived samples.

6.2.6.1. Preparation of Secreted Proteins

In order to detect the proteins of interest by mass spectrometry (MS), the samples were separated on Nu-PAGE gels. The mass spectrometric analysis of keratinase, pectinase and chitinase was achieved using wet NuPAGE® 4-12% Bis- tris acrylamide gel Novex. Nu-PAGE conditions were similar to those for previous Nu-PAGE. One different is loading buffer was MOPS 20X and protein marker was SeeBlue® Plus2 Pre-Stained Standard.

- **Loading buffer (NuPAGE® MOPS SDS Running Buffer (20X)**

NuPAGE® MES SDS Running Buffer and NuPAGE® MOPS SDS Running Buffer both can be used with NuPAGE® Novex® Bis-Tris gels. The use of MOPS buffer allows proteins to run slower than when using MES buffer. The formulation of buffer was 50 mM MOPS, 50 mM Tris Base, 0.1% SDS and 1 mM EDTA, pH 7.7.

- **Standard Protein marker Mixture**

The SeeBlue® Plus2 Pre-Stained Standard (14-191kDa) was purchased from Life Technologies. This marker can be used with NuPAGE® (NuPAGE® 4-12% Bis- tris gel Novex). It contains 10 proteins: eight blue-dyed and two with contrasting colors providing easier band identification. The marker used consisted of Myosin(191 kDa), Phosphorylase B(97 kDa), BSA(64 kDa), Glutamic dehydrogenase(51 kDa), Alcohol dehydrogenase(39kDa), Carbonic anhydrase(28 kDa), Myoglobin- Red(19 kDa) and Lyzozyme(14 kDa).

- **Electrophoresis**

Before identification of candidate bands using MS analysis, the sample run as a soluble sample on a NuPAGE precast. 20 µl of the sample was loaded onto the gel using voltage of 200 for approximately 45min.

6.2.6.2. Liquid chromatography mass spectrometry Analysis (LC-MS/MS)

Mass spectrometry has been generally used to allow identification of proteins using available databases. The criteria for a successful MS analysis are a good match of protein with database which acts as large protein sources. Model LC-MS/MS was used including three steps as follows:

Protein digestion

After several washes of the gel with ultra-high purity water, bands were excised with a clean scalpel and transferred into Eppendorf tubes (Fig. 6.1). Samples were then reduced by 10mM dithiothreitol at 56°C for 1 hour and alkylated using 55mM iodoacetamide 30 min at room temperature in the dark before in- solution tryptic (Sigma-Aldrich) digestion in a final concentration of 50mM ammonium bicarbonate, pH 8 at 37ºC for 16 hours- with a total of 0.4µg of trypsin in a final volume of 70 µl.

LC-MS/MS Analysis

Peptides were then collected and eluted by rounds of incubation with 100% acetonitrile then 0.5%formic acid at 37°C for 15 minutes before being vacuum dried then subsequently being solubilized in suspension (0.1% formic acid, 3% acetonitrile). 40% of the material was injected using a Dionex Ultimate 3000 uHPLC, onto a PepMap100 C18 2cm x75µm I.D. trap column (ThermoFisher Scientific) at 5µL/min in 0.1% formic acid, 2% acetonitrile and 35°C in the column oven and 6°C in the autosampler. The sample was separated over a 36 minute gradient of increasing acetonitrile from 2.4% up to 72%, in 0.1% formic acid using a 15cm PepMap100 C18 analytical column (2µM particle size, 100Å pore size 75µm I.D) (ThermoFisher Scientific) at 250nL/min and 35°C. The mass spectrometer analyser used was a electron transfer dissociation (ETD) (ThermoFisher-Scientific), Orbitrap Elite, equipped with an EasySpray ESI source (ThermoFisher Scientific). Nanospray ionization was carried out at 2.0kV, with the ion transfer capillary at 250°C, and S-lens setting of 60%. MS^1 spectra were acquired at a resolving power of 60,000 with an automatic gain control (AGC) target value of $1x10^6$ ions by the Orbitrap detector, with a range of 350-2000m/z. Following MS^1 analysis the top 20 most abundant precursors were selected for data dependant activation (MS^2 analysis) using collision induced dissociation (CID), with a 10ms activation time and an AGC setting of 10,000 ions in the dual cell linear ion trap on normal scan rate resolution. Precursor ions of single charge were rejected and a 30 second dynamic exclusion window setting was used after a single occurrence of an ion.

Database Searching

The resulting spectra were searched with Mascot (Matrix Science) against the Swissport/Uniprot and NCBInr databases sequentially (with a taxonomy filter of Eubacteria) and using a decoy database within the Proteome Discoverer 1.3 software package

(ThermoFisher Scientific). A mass tolerance of 5ppm was used for precursors and 0.2Da for fragment ions. Full trypsin enzymatic specificity was required with up to 2 missed cleavages permitted, carbamidomethylation of cysteine was specified as a fixed modification, oxidation of methionine and acetylation of protein N-terminus were characterized as variable modifications. False discovery rates (FDRs) were set at 1% (strict) and 5% (relaxed) by Peptide Validator (workflow node within Proteome Discoverer) and were used to distribute the confidence indicators for the peptide spectral matches. Proteins required a minimum of two peptides with a 95% confidence interval or above in order to be reported.

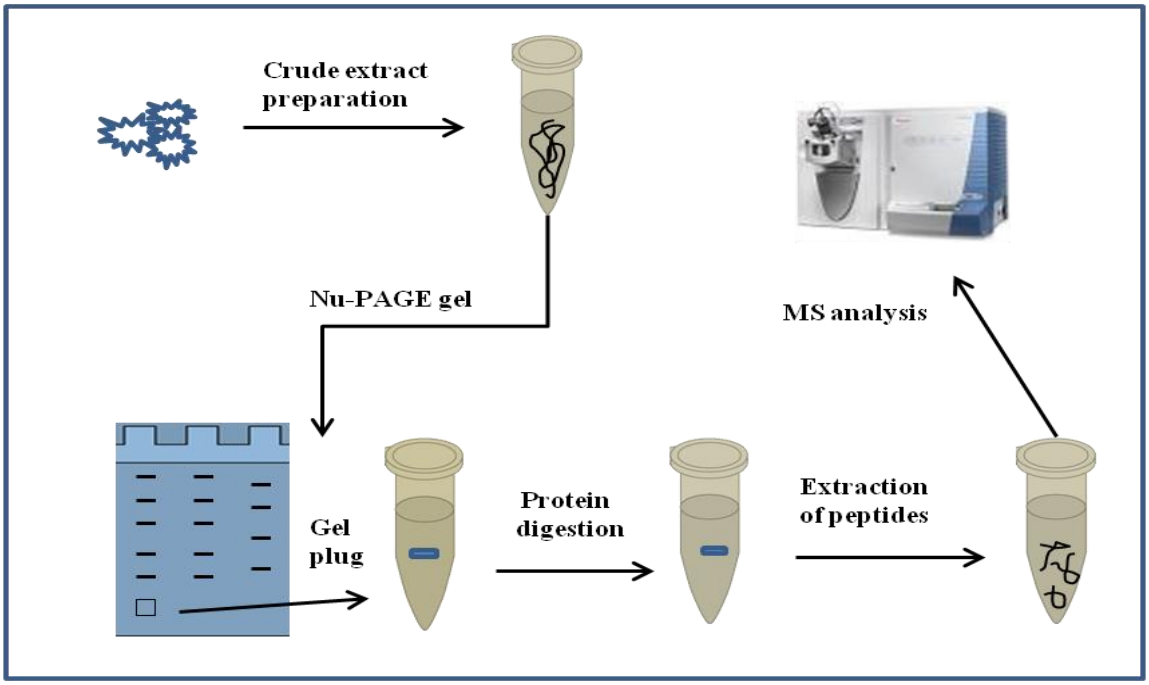


Figure 6.1: The main steps of mass spectrometry.

6.3. Results and Discussion

6.3.1. Analysis of alginate lyase according to the protein marker

A small number of alginate lyases have been purified from marine bacteria, but others have been tested as crude extract (partially purified states) from marine and terrestrial bacteria (Sawabe *et. al.*, 1992). Alginate lyase was successfully partially purified here using ammonium sulphate as crude extract. The partial purified enzyme was confirmed on SDS-PAGE. SDS-PAGE was allowed to recognize the band and show the alginate lyase has a single band estimated as 32 kDa (Fig.6. 2). The molecular mass of alginate lyase found was similar to most *Pseudomonas* alginases.The present findings support the study of Muramatsu and Sogi, (1990) study which concluded that alginate lyases from *Pseudomonas sp.*,

partially purified from culture medium which gave the MWt of the extracellular enzyme was approximately 32 kDa. Several studies show similar results to the ones given here for bacterial alginate lyases. Sawabe *et al*. (1992,1997) found that purified alginate lyase from the culture medium of *Alteromonas sp. H-4* have a MWt of 32 kDa using SDS-PAGE, which is in good agreement with the results of the present study. However, molecular weight of alginate lyase was shown to be 37 kDa in *P. aeruginosa* (Glonti *et al*., 2010) . The present finding was consistent with the finding of Schiller *et al*. (1993), who reported that *P. aeruginosa* alginase was estimated to be 39 kDa.

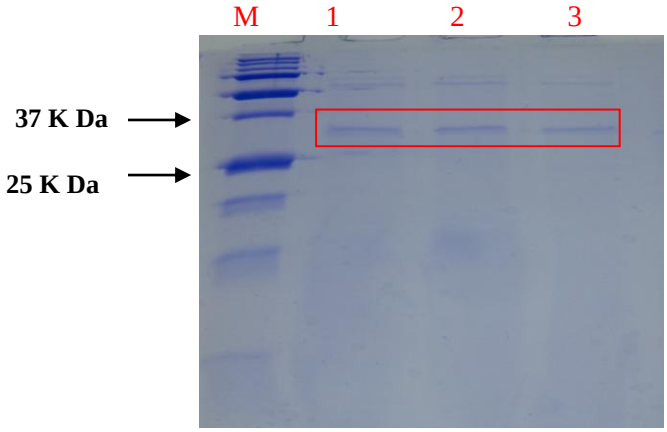


Figure 6.2: SDS-PAGE of Partially purified alginase from *P.aeruginosa* ATCC 27853.

6.3.2. Measurement of keratinase, pectinase and chitinase activities

The results shown in Figure 6.3, 6.4 and 6.5 show the activity of the three enzymes measured using Bio-Rad regent based on Bradford methods. Figure 6.3 and 6.4; show that the activity of chitinase and keratinase from crude extract was higher than the supernatant. However, there was a considerable decrease in pectinase activity in crude extract compared to supernatant (Fig. 6.5).

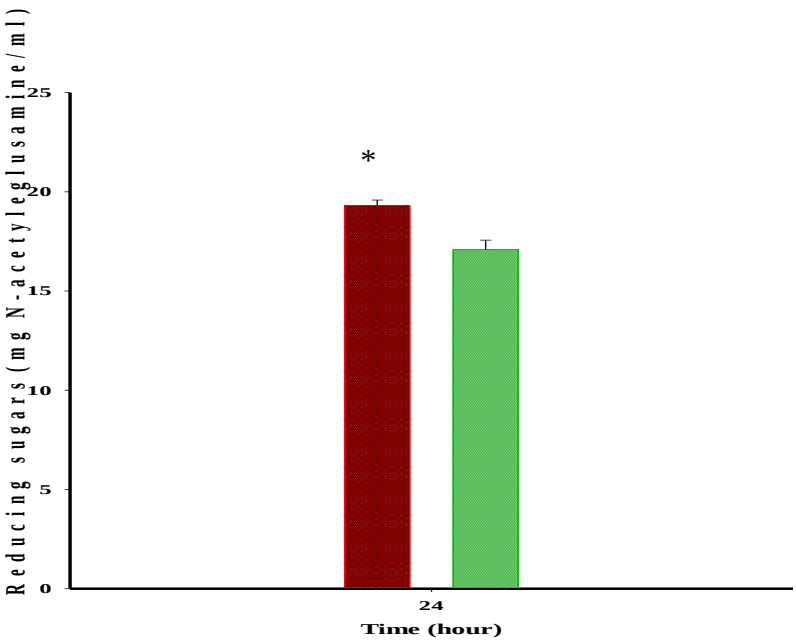


Figure 6.3: Activity of chitinase of *B. cereus*; crude extracts ( ) and supernatant ( ). Means of triplicates (±) standard error. * significant difference from the crude extract and supernatant ($P < 0.05$).

It can be clearly seen from the Figures that high enzyme activity in the crude extracts was shown by keratinase and chitinase and low enzyme activity was observed in pectinase. A possible explanation for this is based on losses of enzyme activity due to using of 80% ammonium sulphate.

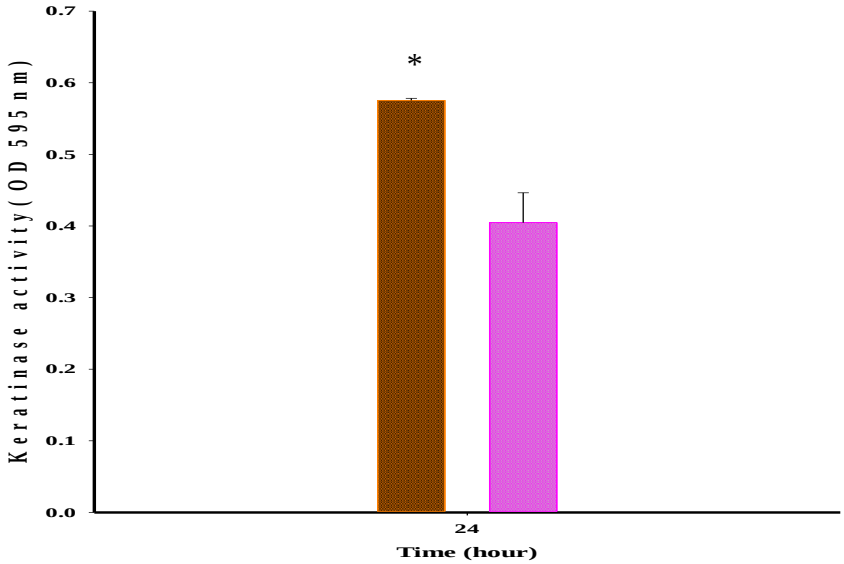


Figure 6.4: Activity of keratinase of *B. licheniformis*; crude extracts ( ) and supernatant ( ). * Significant difference from the crude extract and supernatant ($P < 0.05$).

As indicated by Wen *et al*., (2002), in general methods the culture is handled with 85% $(NH_4)_2SO_4$ to precipitate the protein as crude extract.

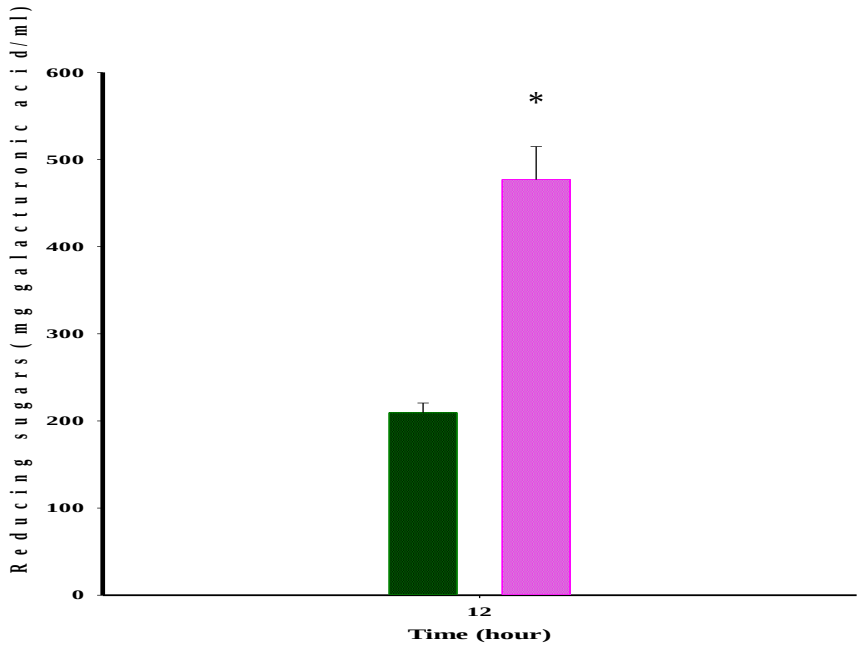


Figure 6.5: Activity of pectinase of *B. megaterium*; crude extracts ( ) and supernatant ( ). Means of triplicates (±) standard error. * significant difference from the crude extract and supernatant ($P < 0.05$).

Unfortunately, only a limited amount of protein can be salted by 85% saturation $(NH_4)_2SO_4$. Subsequently, about 75% of the total enzyme activity was lost in this step. Moreover, there is variation in enzyme solubility, some enzyme remain in solution and ammonium sulphate unable to precipitate them. It can be concluded that decreases pectinase activity might have occurred during the precipitation process.

6.3.3. Determination of enzyme concentration

The results from Bio-Rad assay showed that the concentrations of enzymes were between 0.2-0.5 mg/ml. The concentration of protein measured in chitinase was found to be significantly lower than keratinase and pectinase. The highest concentration was detected in keratinase produced by *Bacillus licheniformis* and pectinase produced by *B.megaterium* (0.5 and 0.45mg/ ml respectively). However the lowest concentration was observed in the chitinase produced by *B. cereus* (0.2 mg/ml).

6.3.4. Partial characterization of keratinase, pectinase and chitinase

The enzymes were partially purified by the addition of solid ammonium sulphate to the supernatant. The fractions were loaded onto the SDS-PAGE. Initially, the extracellular proteins were observed by SDS-PAGE. The number of bands observed in NuPAGE were seen to be different. The proteins from *Bacillus licheniformis*, *B.megaterium* and *B.cereus* resulted in one or two major protein bands and a larger number of minor components as seen using Nu-PAGE (Fig. 6.6).

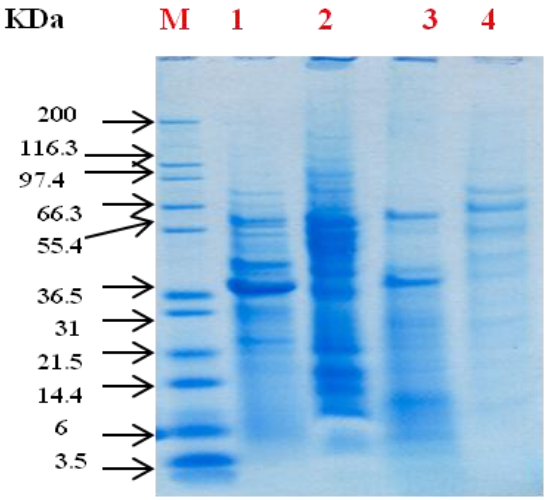


Figure 6.6: Nu-PAGE of partially purified enzymes. Lane 1: keratinase from *Bacillus licheniformis*, Lane 3*:* pectinase from *B.megaterium*. Lane 4: chitinase from *B.cereus* strain BS2.

According to the standard protein marker, the size of the major band of keratinase from *B. licheniformis* was found to be around 36.5kDa which is the same as pectinase from *B.megaterium*, meanwhile the major band of chitinase from *B.cereus* were estimated to be 60kDa.

6.3.5. Identification of enzymes using mass spectrometric LC-MS/MS analysis

The use of mass spectrometry-based proteomics as identification of proteins has become more important in recent years. It has been generally used to study identifying hundreds of proteins in only one experiment. During LC-MS/MS analyses, massive number of peptides could be hit consistently. However, a low number of peptide ions might be missed in one run. The use of collective data from repeat runs lead to the identification of different peptides (Mbeunkui *et al*., 2007). To demonstrate the potential of LC-MS/MS and its suitability for application, the bands from the gel were investigated.

6.3.5.1. Identification of chitinase

Two candidate bands from Nu-PAGE were cut, prepared and analyzed by MS/MS (Fig.6.8) in order to provide reliable proteomic profiling. Crude extract from *B. cereus* strain BS2, resulted in two major protein bands and a larger number of minor components by Nu-PAGE (Fig. 6.7). Based on the LC-MS/MS analysis, the band C1 corresponded to chitinase. It was successfully positive for chitinase, when the data were searched against the NCBI database. This was supported by the over 90% coverage of proteins on the lists in the combined data sets. However, the other major band C2 did not show chitinase. Interestingly, however, they showed the presence of very large protein in C2 and very small protein in C1; C2 was not the target enzyme. Consequently, this is confirmed that chitinase C1 and C2 must be different.

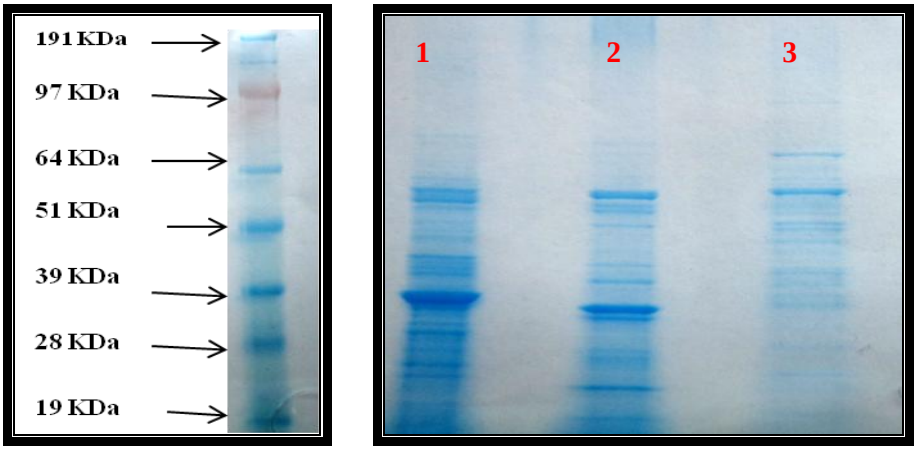


Figure 6.7: SDS-PAGE before cutting the bands.

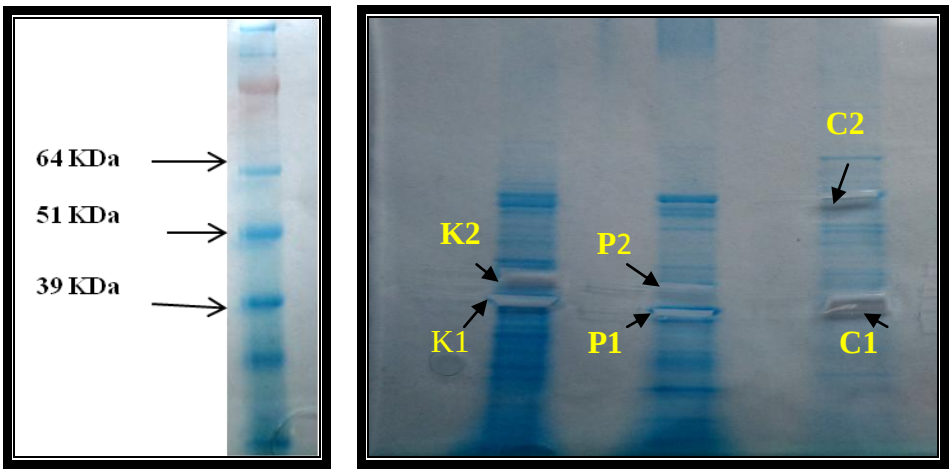


Figure 6.8: The location of candidate bands.

As proof of positivity, a search of protein sequence databases shows that there is a significant similarity of *B.cereus* strain BS2 chitinase with known proteins. As the aim of this research was to identify highly secreted chitinase, it was focused on the top ten proteins and chitinasae located in the fifth position of the list. A list of more significant proteins and analysis of fraction C1 is shown in Table 6.4, showing charge with the number of peptide sequence.

Table 6.4: Summary of the peptides and proteins identified in C1 using the LC column.

Peptide Sequence	Protein group	Protein Group Accessions	Charge	Proteins	Mass of protein (Da)
STVEFSPVYGTDAEFK	1	222097180	2	5	1776.83208
VVLSIGGQNGVVLLPDNAAK	1	222097180	2	5	1964.11333
SDQVMIGLPAAPAAAPSGGYISPTEmK	1	222097180	2	5	2675.30425
LSNESGYPAFR	1	222097180	2	5	1240.59319
SDQVMIGLPAAPAAAPSGGYISPTEMK	1	222097180	2	5	2659.30522
FINSIQSLIDK	1	222097180	2	5	1277.70989
KALDYIIK	1	222097180	2	19	963.58672

In order to determine the molecular weight from the right band, the band corresponding to chitinase (C1) appeared on Nu-PAGE with molecular weight approximately 39 kDa using mass spectrometric. The molecular weight obtained from the gel (60 kDa) was compared with the molecular weight obtained from mass spectrometric (39 kDa) which shows that the determination of molecular weight of protein using mass spectrometric is more accurate than using the gel approach. The present findings support Chang *et al.*, (2010) study which concluded that molecular masses of *Bacillus* chitinases ranging from 35 to 89 kDa. The molecular mass of chitinase from the present study is also similar to the chitinase reported by Chang *et al*., (2003); 38kDa for *Bacillus cereus* and *Bacillus circulans* release two chitinase 38 and 39 kDa.

6.3.5.2. Identification of keratinase and pectinase

In this study the MS analysis was assessed by cutting out the candidate bands taken from the gel and then digesting them using trypsin. MS analysis of keratinase and pectinase based

on cutting of candidate bands resulted in no significant similarity with the database of keratinase and pectinase; for unknown reasons that could be related to many factors. A reasonable explanation is that the candidate bands were not the right bands. These results suggest that the enzymes may have acted on different bands of the gel. Several impact factors might have contributed to unsuccessful peptide detection in LC-MS/MS, For example:

- **Poor signal-to-noise ratios**

The presence of a large numbers of peptides in the digested protein mixture produces problems with separation. Problems with protein detection may have occurred when only one cycle of mass spectrometry was achieved for the protein. In addition, poor signal-to-noise ratios are obtained or incomplete groups of ions prevent unmistakable confirmation of the search result (McCormack *et al*., 1997).

- **limited scanning capacity**

A signal gel plug approximately 1mm wide might include 40 proteins and to result in at least 2000 peptides in part of proteolysis of this slice. This degree of complexity in the sample is likely to make it impossible to identify all the proteins found by LC-MS/MS due to the limited ability (limited scanning capacity) (Baker *et al.*, 2007). Undetectable samples in the analysis could be related to high-sensitivity applications to a single setup of the instrument. This sensitivity is due to the material in the column and not from protein material in the gel (Mann *et al.*, 2001). It appears that the major band is not a good indicator for the target protein and further efforts will be need to be made to purify and optimize the conditions of protein, including, loading, band cutting and enzyme purification. These results recommended the crude extract must be further purified in order to obtain a single band.

CHAPTER 7

CHAPTER 7

GENERAL DISCUSSION

Huge amounts of organic and inorganic substrates are discarded and pollute soils and the water courses every year. Biological treatment of such wastes tend to be cheaper and more environmentally friendly than chemical and physical treatments (Mrayyan and Battikhi, 2005). The degradation of complex substrates is required by microorganisms in order to obtain energy and nutrients for their growth and survival. The activity of extracellular enzymes is associated with the rate of degradation (Hernández and Hobbie, 2010), and bacteria and fungi play important roles in this process. The aim of this work was to study the degradation of four complex substrates, namely keratin, pectin, alginate and chitin, by bacteria and fungi as a safe solution to the environment problems caused by their release into the environment. An additional aim was to determine the activities of the four enzymes involved in these degradative processes. The current study also investigated the breakdown of chitin, pectin, keratin and alginate in soil in relation to nitrification, ammonium production and sulphur oxidation.

The number of keratinophylic fungi isolated using hair as baits via a hair-baiting technique (HBT) were larger than that achieved using wool as baits, showing that hair is a more effective keratin substrate for use in the isolation of keratinophylic fungi. A small number of keratinophylic fungi were isolated using feather which proved not to be a favourable isolation substrate for keratinophylic fungi. Solid media supported by commercial substrate (keratin azure) as a source of carbon and nitrogen promoted keratinase production for keratinolytic fungi and keratinolytic bacteria. The following keratinolytic fungi were isolated: *Acremonium strictum, Penicillium verruculosum* and *Trichophyton mentagrophytes.* Surprisingly, *Penicillium verruculosum* (non-dermatophytes) was seen to be more active keratinase produce than was *Trichophyton mentagrophytes* which is a well-known dermatophyte. This finding shows that some non-dermatophyte fungi have higher keratinolytic potential than do some dermatophytes. Again surprisingly, the addition of carbon and nitrogen source did not increase keratinase production and the production of this enzyme was inhibited by the addition of sucrose and peptone as source of carbon and nitrogen. The findings also confirmed that there is no connection between fungal biomass and the degradation rates of keratin azure. Scanning microscopy studies showed degradation and structural change of hair and wool during the surface erosion and this phase was obvious. The

addition of pectin, esterified potassium salt, from citrus fruit to agricultural soil repressed the nitrate production. The reason was not clear. Presumably, the microorganisms did not prefer to use this substrate. The rate of ammonium production increased only after 2 weeks and the dominance in ammonium production was associated to the soil without pectin (control).

The results presented here show that pectin exhibits antibacterial activity against *E.coli*, *Staph.aureus* and *Staph.aureus* MRSA3 (5% pectin). However, pectin had no effect against *Pseudomonas aeruginosa.* These findings suggest that pectin has a narrow range of antibacterial activity and exhibits selective toxicity against *E.coli*, *Staph.aureus* and *Staph.aureus* MRSA3.

The marine fungi isolated from *Fucus* and *Laminaria* as marine habitats showed good growth in artificial medium (B and K plates) using seawater, and bacterial isolates from marine sand grew well on sodium alginate plates and Tryptic soy agar medium but did not grow on nutrient agar. This suggests that marine bacteria require rich medium to grow. The marine fungi isolated from *Laminaria* were, *Penicillium chrysogenum, Penicillium crustosum* and *Dendraphiella salina*. All isolates from *Laminaria* were more active in alginate degradation in sodium alginate medium 0.5% NaCl than those obtained from *Fucus.* This study has also shown that all marine bacteria isolated from marine habitat were alginolytic and released alginate lyase in solid medium. Some non marine bacteria, i.e. *P. aeruginosa* , *C.necator*, *B. megaterium* and *Rhizobium sp.* were also able to use alginate as a source of carbon. Aqueous ruthenium red stain (0.05% wt/vol) was an excellent stain to detect alginase activity in plates. In addition, calcium- and strontium-free artificial sea water (NaPYNS) was as good component for use in the preparation of alginate medium. Interestingly, none of the bacteria and fungi (marine or terrestrial) could breakdown alginate when artificial seawater (3% NaCl) was used. Consequently, this study has found that high salinity was not essential for marine bacteria and fungi and it inhibited alginate lyase production. The most obvious finding to emerge from this study is that *D. salina* produced non-reducing sugars when grown on sodium alginate medium and that growth brought about a major change in medium-pH. The results also showed that *Alternaria tenuissima* produced reducing ends (glucose) into sodium alginate medium indicating the production of alginase. Interestingly, *P. aeruginosa* produced both non-reducing sugars and reducing ends in alginate medium leads to alginate degradation. Freshly collected *Fucus* and *Laminaria* released nitrate when added to soil, with nitrate production being higher in soil amended with *Laminaria* than amended with *Fucus.* The same action was obtained from ammonium production. As a result, the soil amended

with *Laminaria* was perfect soil to nitrate and ammonium production. Nitrate production in soil amended with commercial seaweed was low. However, ammonium production and sulphate oxidation was substantial. The following conclusion can be drawn from the present study; nitrification, ammonium production and sulphur oxidation occurred in agricultural soil amended with fresh or commercial fertilizer seaweed.

In relation to chitin the results given in Chapter 5 show that the fungi, isolated from soil amended with chitosan, were mostly *Trichoderma* species, e.g. *Trichoderma harzianum* and *Trichoderma tawa.* The findings show that acid hydrolysis of chitin produced colloidal chitin which supported fungal and bacterial growth. Using a media test based on the use of bromocresol purple stain, all *Trichoderma* species were shown to exhibit chitinolytic activity (by production of a purple zone). *Trichoderma harzianum* produced a large purple zone, while *M. hiemalis* produced a small zone due to lower chitinolytic activity. These findings provide evidence that *P.aeruginosa, E.coli, C. necator* and *Rhizobium sp* are active chitinase producers. In contrast, *Staph. aureus* and *B. thuringiensis* did not hydrolysis chitin. The present study shows that in Yeast-nitrogen base (YNB) medium, *Trichoderma harzianum* and *Mucor hiemalis* produced chitinase as expressed by the production of N-acetyglucosamine (reducing sugars) using DNS methods. High levels of chitinase were formed in the early growth stages by *Trichoderma harzianum.* However, *M. hiemalis* produced high amount of GLcNAc at only the 5 days sample point; alkalinization of the medium was observed in both cases. The findings show that *B.cereus* releases free glucose as end products from chitin degradation, i.e. that *B.cereus* has chitinolytic activity. Nitrate levels in agricultural soil amended with chitosan was considerable and the nitrate production occurred in all incubation periods, related to the fact that chitin is composed of amino sugars which are hydrolysed to ammonium. The concentration of ammonium produced from decomposition of chitosan was low and the levels of ammonium with the highest values occurring at week one.

Finally, the isolation (alginate lyase) from *P.aeruginosa* ATCC 27853 was achieved successfully using two saturations of ammonium sulphate. The protein was not further purified (crude extract) and was estimated as 32kDa by (SDS-PAGE). Proteins (enzymes) also extracted from *Bacillus licheniformis* (keratinase), *B.megaterium* (pectinase) and *B.cereus* (chitinase) resulted in larger number of minor components and small number of major bands. These results suggest that the nature of the proteins need to be confirmed and identified for analysis. LC-MS/MS Analysis of chitinase showed that the band from chitinase lane corresponded to chitinase. The study also showed that the chitinase of *B. cereus* BS2,

estimated using mass spectrometry, had a MWt of 39kDa. Keratinase and pectinase based on cutting bands resulted no similarity with the database was observed. A sensible justification is that the candidate band was not the right band. These results suggested that the identification of these enzymes need considerable work to prove.

Suggestions for further study

Recently, the degradation of keratin, pectin, alginate and chitin has become of major concern in relation to the bioremediation of polluted environments. In the past years, the bacteria and fungi have attracted attention based on the fact that they are active degraders of these complex substrates. The findings given here in this Thesis could be extended as follows:

(i) Use transmission electron microscopy (TEM) in order to determine the phases of complex substrate breakdown, particularly in relation to keratin degradation.

(ii) To use more specific methods to isolate the four proteins and study the enzymes.

(iii) To purify the proteins after extraction before conducting LC-MS/MS Analysis. Further investigation and experimentation for enzymes purification is also strongly recommended.

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APPENDIX

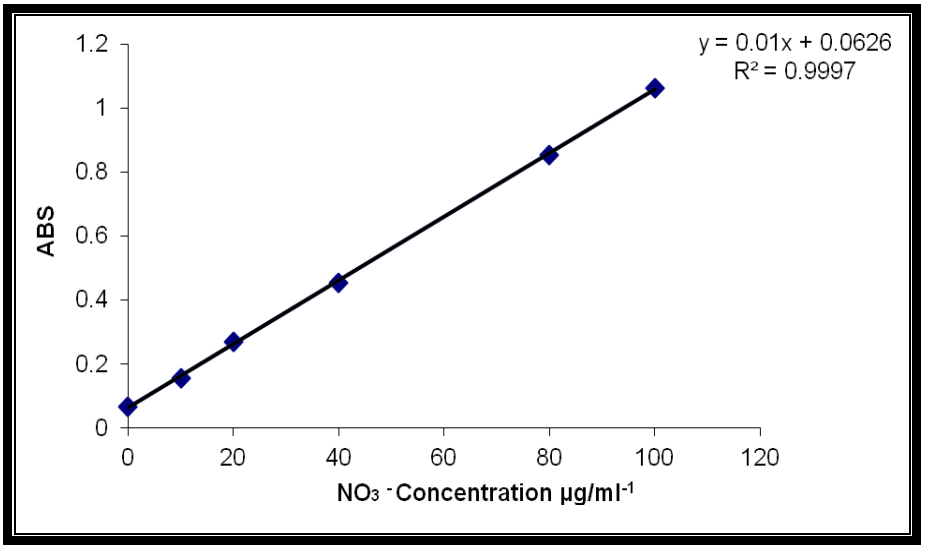
APPENDIX

Appendix A

Standard curves preparation

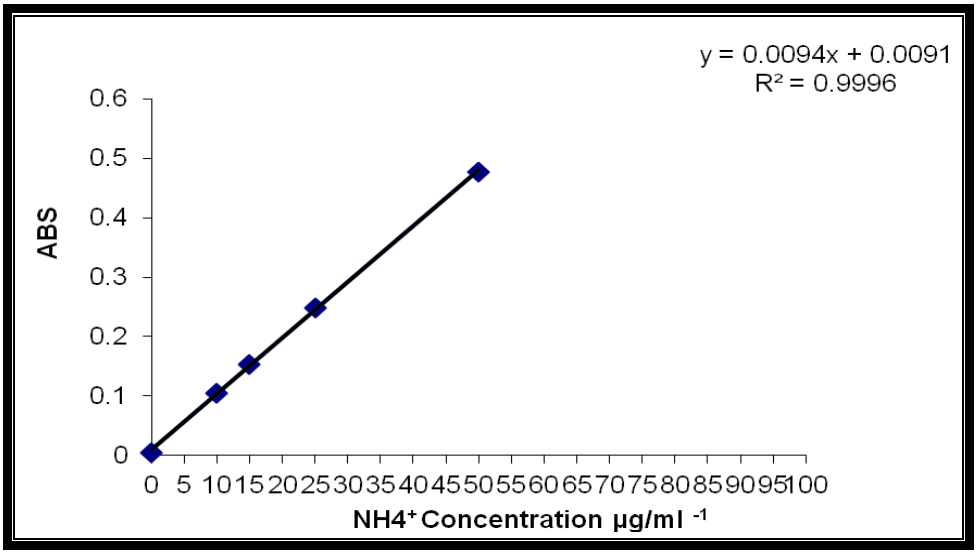
● Standard curve for nitrate

It is necessary to create a nitrate standard curve. To obtain 1000µg $NO3^-$-N ml^{-1}, 1.37 g of sodium nitrate was dissolved in 1000 ml dH_2O. In order to the solution equivalent 100µ g / NO_3^--N ml^{-1} (10 times dilution), 10 ml of sodium nitrate solution was mixed with 90 ml of $d_{H2}O$. Serial dilutions from previous solution were made with dH_2O to obtain 0, 10,25,50,75, and 100 µg NO_3^--Nml^{-1}. chromotropic acid methods was performed for all dilutions to evaluate nitrate ions (Sims and Jackson, 1971).



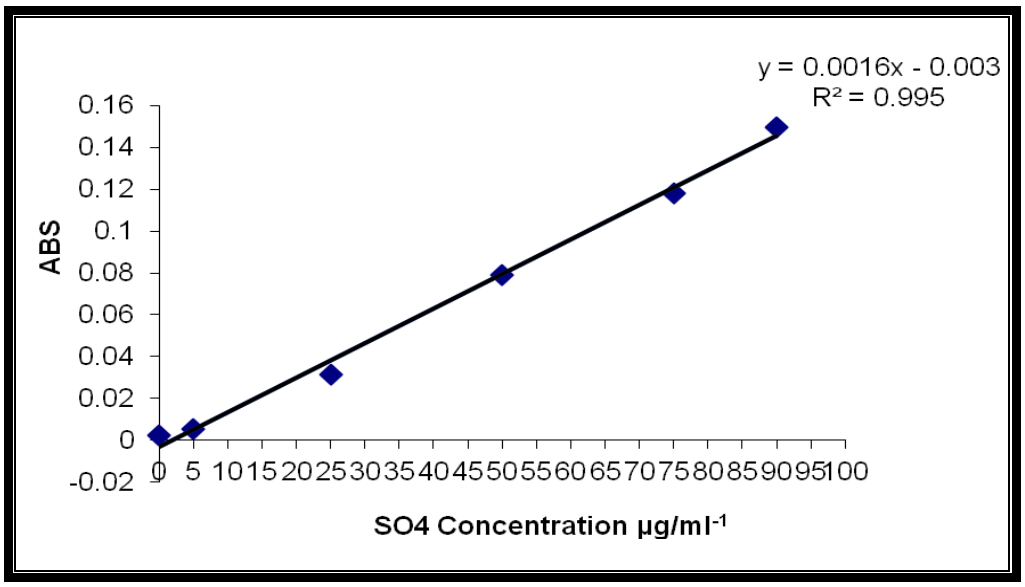
● Standard curve for ammonium

A calibration curve for ammonium ions was performed by dissolving 3.66 g of $(NH_4)_2SO_4$ ammonium sulphate in 1000 ml dH_2O which equal 1000µg NH_4^+-Nml^{-1}. 10 ml ammonium sulphate solution was mixed with 90 ml of dH_2O (10 times dilution) to obtain 100µ g / $NH4^+$-Nml^{-1}. Serial dilutions from previous solution were made with dH2O to obtain 0, 10, 15, 25, and 50 µg NH_4^+-N ml^{-1}. Indophenol blue method was performed for all dilutions to evaluate ammonium ions (Wainwright and Pugh, 1973).



● Standard curve for sulphate-S

A calibration curve for sulphate ions was performed by dissolving 1.47 g of sodium sulphate (Na_2SO_4) in 100 ml dH2O which equal 1000µg SO_4^{2-}-Sml^{-1}. 10 ml of sodium sulphate solution was mixed with 90 ml of dH_2O (10 times dilution) to obtain 100µ g / SO_4^{-2}-S ml^{-1}. Serial dilutions from previous solution were made with dH_2O to obtain 0, 5, 25, 50, 75 and 90 µg SO_4^{2-}-Sml-1. Turbidimetric methods were performed for all dilutions to evaluate sulphate-S ions (Hesse, 1971).



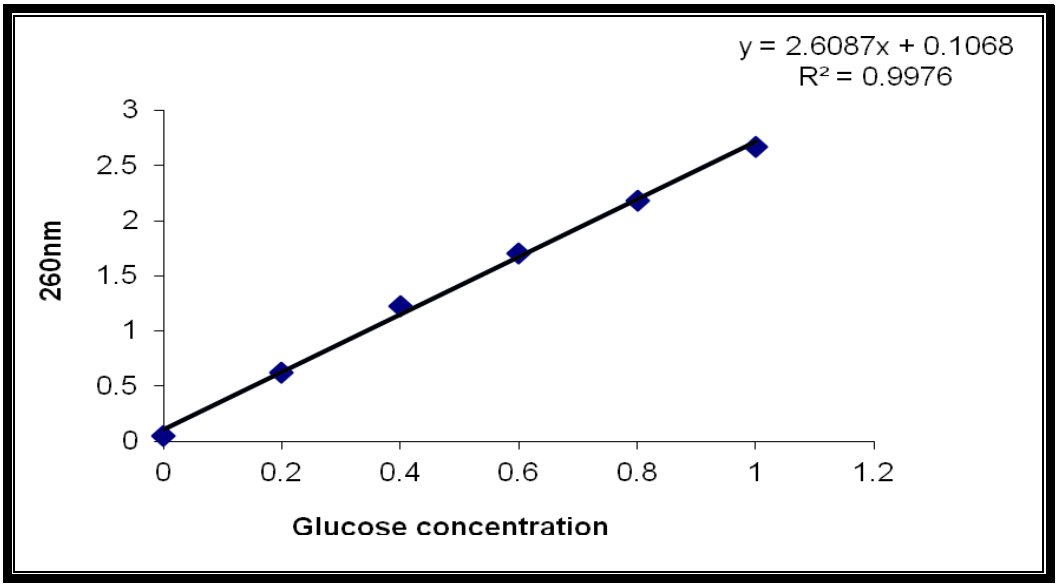
● **Standard curve for glucose using the Nelson methods at 620nm**

(i) Preparation of glucose solution stock

100mg D (+) glucose was dissolved in 100ml distilled water

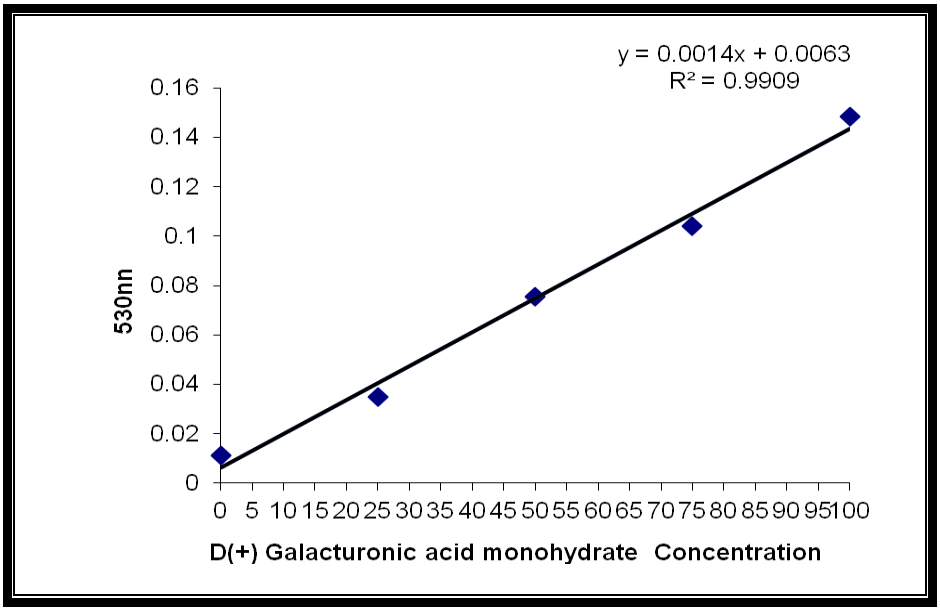
(iii) **Preparation of glucose solution stock**

10ml of glucose solution was taken and diluted up to 100ml with distilled water to obtain concentration 100µg/ml. Serial dilution 0,0.2,0.4,0.6,0.8 and 0.10 ml were taken from working standard solution and put in test tubes to obtain 20%,40%,60%,80 %and %100.Then the volume was adjusted up to 2ml in each tube. 1ml of alkaline copper tartrate was added into each tube then the mixture was boiled for 10min. The tube was cooled then 1 ml of arsenomolybdate regent was added, and the blue colour after 10 min was read at 620nm using 2ml distilled water as blank.



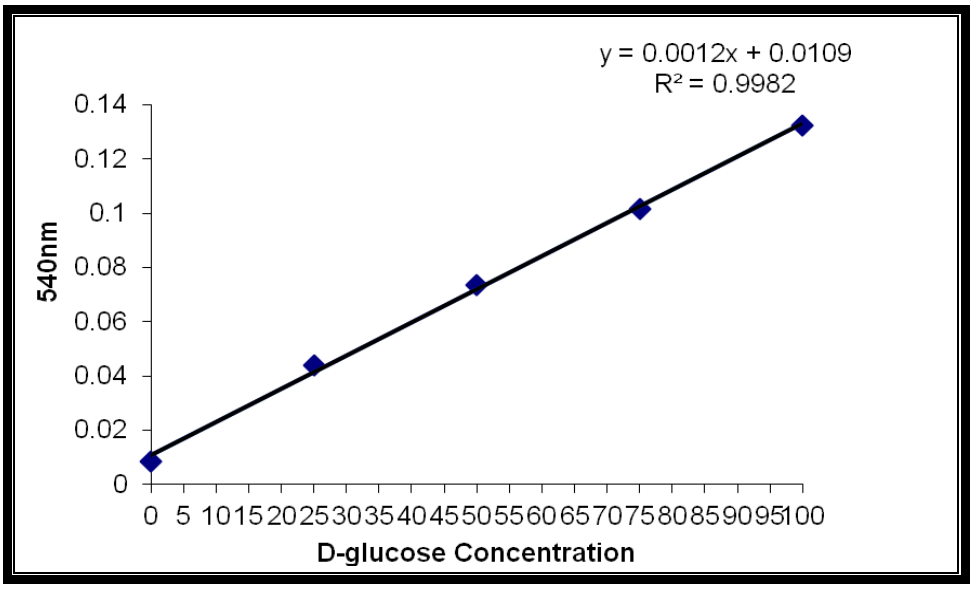
● **Standard curve for monogalacturonic acid using DNS methods at 530nm**

A calibration curve for monogalacturonic acid was prepared by dissolving 0, 1.2, 2.5, 3.8, and 5 mg in 5ml of dH_2O to produce following dilutions: 0,15,50,75 and 100. 200µl from each dilution was added to 400 µl of DNS regent then the mixture boiled at 100C for 10min. After cooling the solution was diluted by dH2O upto14 ml and the absorbance was read at 530 nm (Taylor and Secor).



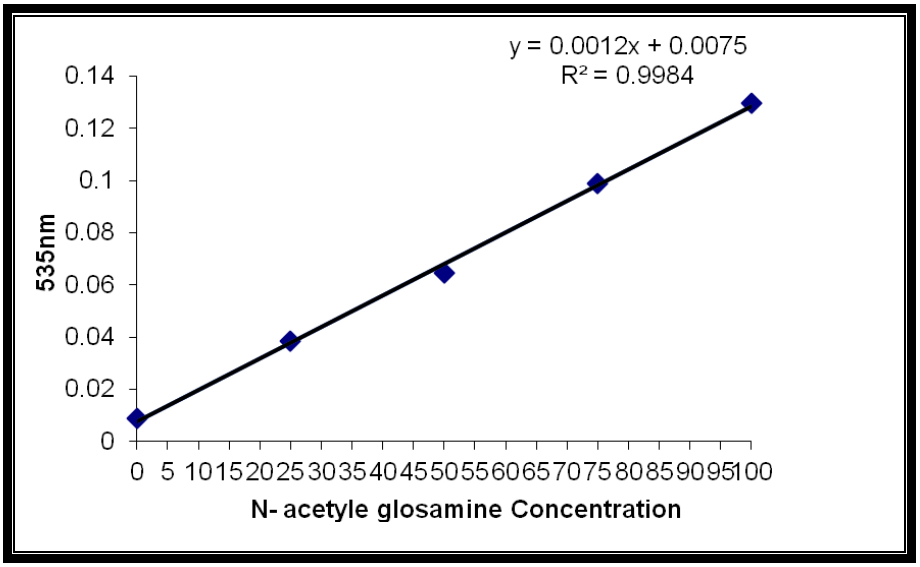
● Standard curve for glucose using DNS methods at 540nm

A calibration curve for D- glucose using DNS methods was performed by dissolving 0, 1.2, 2.5, 3.8, and 5 mg in 5ml of dH_2O.The same procedure used in galacturonic acid was done. The absorbance was read at 540nm.



● Standard curve for N-acetyl glucosamine

A calibration curve for N-acetylglucosamine using DNS methods was performed by dissolving 0, 1.2, 2.5, 3.8, and 5 mg in 5ml of dH_2O.The same procedure used in galacturonic acid was done. The absorbance was read at 535nm.


y = 0.0012x + 0.0075
R² = 0.9984
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
535nm
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
N- acetyle glosamine Concentration

Appendix B

● Phylogenetic analysis of fungi related to keratin

```
>gb|HQ630346.1|  Mortierella amoeboidea strain CBS 889.72 internal transcribed
spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete
sequence; and internal transcribed spacer 2, partial sequence
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 Identities = 589/594 (99%), Gaps = 3/594 (1%)
 Strand=Plus/Minus

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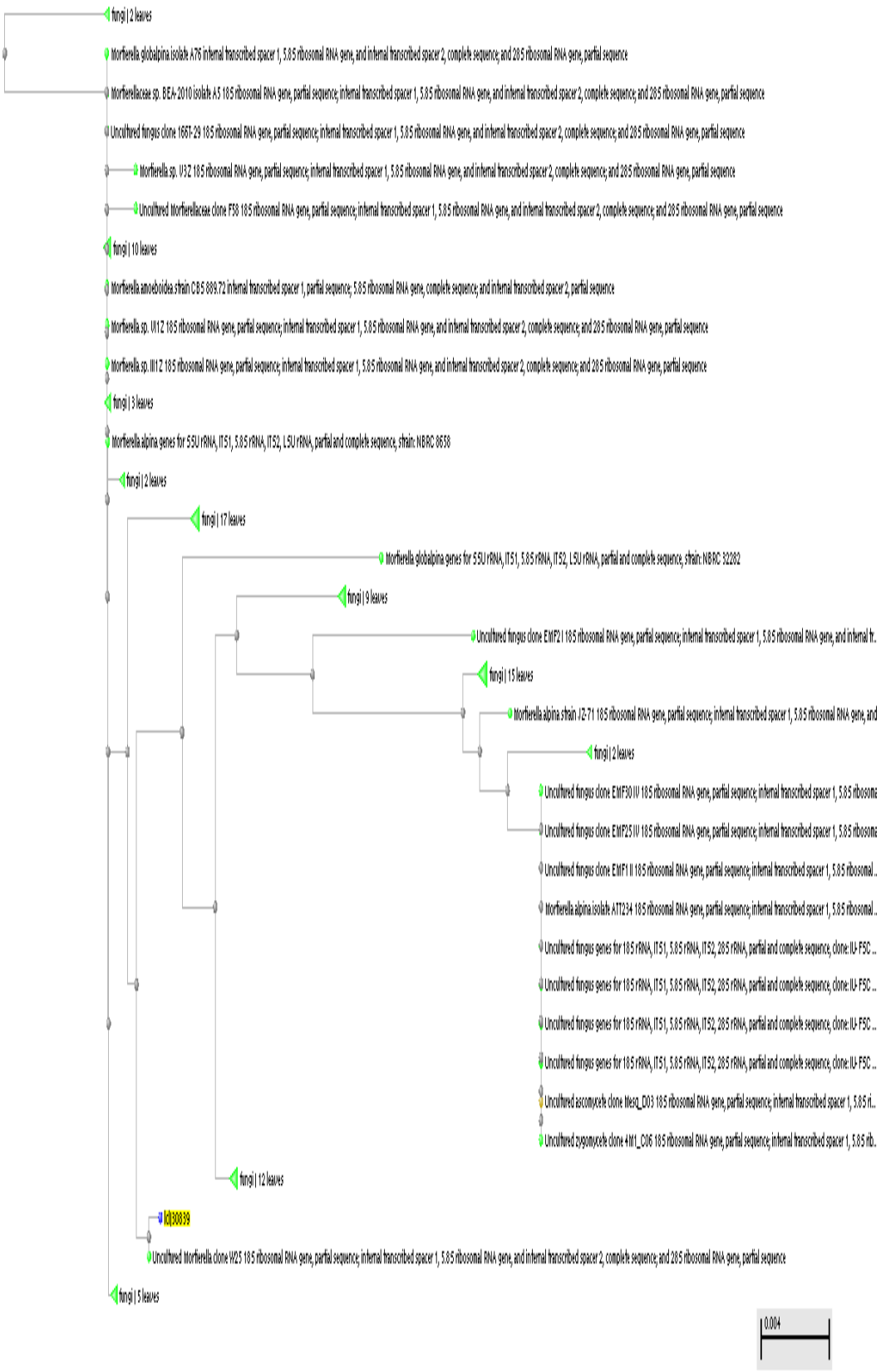
fungi | 2 leaves
Mortierella globalpina isolate A76 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Mortierellaceae sp. BEA-2010 isolate A5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured fungus clone 1661-29 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Mortierella sp. W32 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured Mortierellaceae clone FS8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
fungi | 10 leaves
Mortierella amoeboidea strain CBS 889.72 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Mortierella sp. W12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Mortierella sp. W12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
fungi | 3 leaves
Mortierella alpina genes for 5SU rRNA, ITS1, 5.8S rRNA, ITS2, LSU rRNA, partial and complete sequence, strain: NBRC 8558
fungi | 2 leaves
fungi | 17 leaves
Mortierella globalpina genes for 5SU rRNA, ITS1, 5.8S rRNA, ITS2, LSU rRNA, partial and complete sequence, strain: NBRC 32282
fungi | 9 leaves
Uncultured fungus clone EMF21 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr..
fungi | 15 leaves
Mortierella alpina strain J2-71 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and
fungi | 2 leaves
Uncultured fungus clone EMF30 IV 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosoma
Uncultured fungus clone EMF25 IV 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosoma
Uncultured fungus clone EMF1 II 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal..
Mortierella alpina isolate AT234 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal..
Uncultured fungus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, clone: IU-FSC ..
Uncultured fungus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, clone: IU-FSC ..
Uncultured fungus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, clone: IU-FSC ..
Uncultured fungus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, clone: IU-FSC ..
Uncultured ascomycete clone Mesq_D03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ri..
Uncultured zygomycete clone 4M1_C06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rib..
fungi | 12 leaves
KU180838
Uncultured Mortierella clone W25 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
fungi | 5 leaves
0.004

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Strand=Plus/Minus

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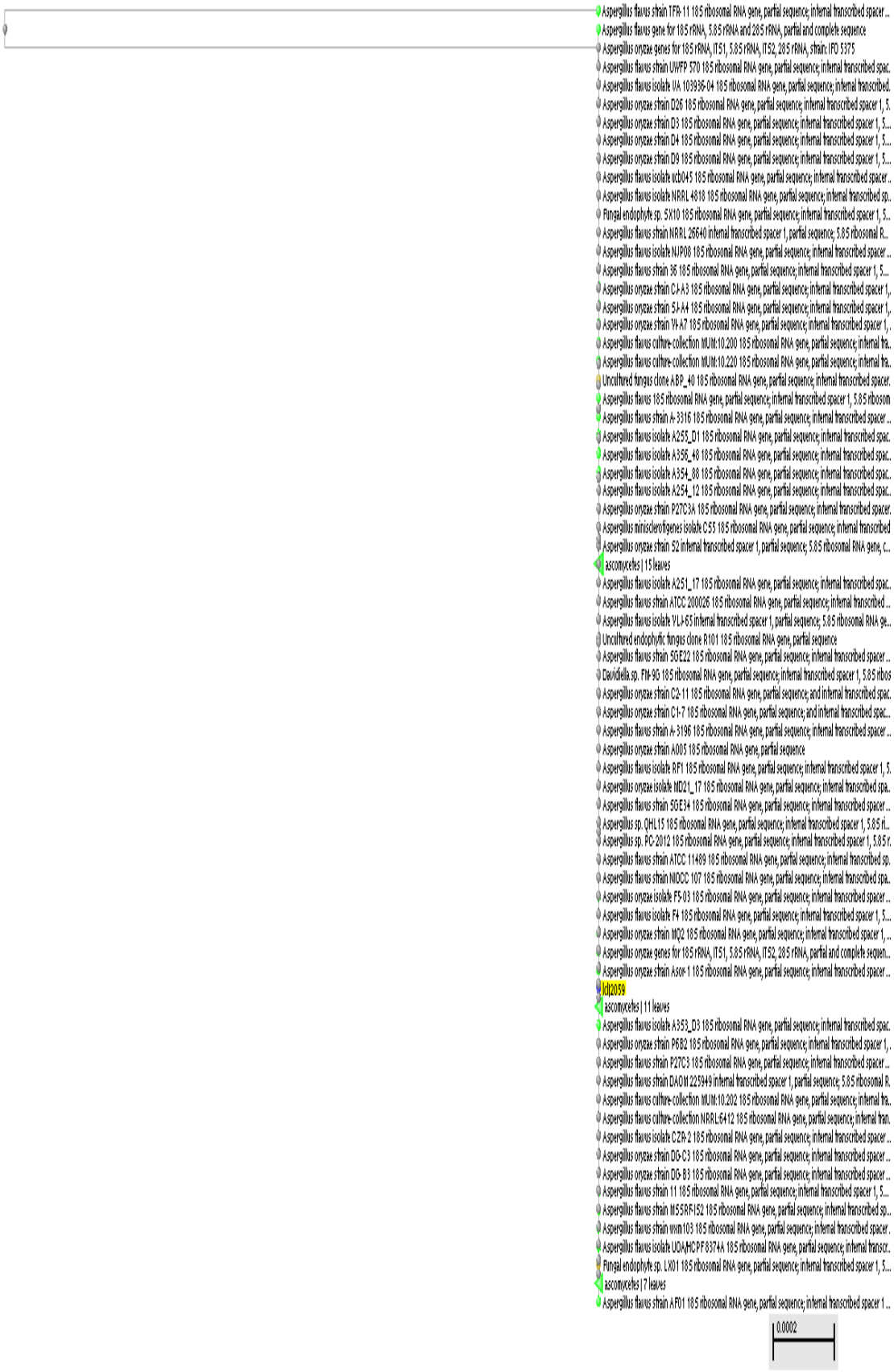
Aspergillus flavus strain TFR-11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
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Aspergillus oryzae genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, strain: IFO 5375
Aspergillus flavus strain UWFP 570 18S ribosomal RNA gene, partial sequence; internal transcribed spac.
Aspergillus flavus isolate IIA 103936-04 18S ribosomal RNA gene, partial sequence; internal transcribed.
Aspergillus oryzae strain D26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.
Aspergillus oryzae strain D3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus oryzae strain D4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus oryzae strain D9 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus flavus isolate ucb045 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ..
Aspergillus flavus isolate NRRL 4818 18S ribosomal RNA gene, partial sequence; internal transcribed sp..
Fungal endophyte sp. SX10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus flavus strain NRRL 26640 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...
Aspergillus flavus isolate NJP08 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ..
Aspergillus flavus strain 36 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus oryzae strain CA-A3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,
Aspergillus oryzae strain SA-A4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,
Aspergillus oryzae strain YA-A7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,.
Aspergillus flavus culture-collection MUM:10.200 18S ribosomal RNA gene, partial sequence; internal tra..
Aspergillus flavus culture-collection MUM:10.220 18S ribosomal RNA gene, partial sequence; internal tra..
Uncultured fungus clone ABP_40 18S ribosomal RNA gene, partial sequence; internal transcribed spacer.
Aspergillus flavus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosom
Aspergillus flavus strain A-3316 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ..
Aspergillus flavus isolate A255_D1 18S ribosomal RNA gene, partial sequence; internal transcribed spac.
Aspergillus flavus isolate A356_48 18S ribosomal RNA gene, partial sequence; internal transcribed spac..
Aspergillus flavus isolate A354_88 18S ribosomal RNA gene, partial sequence; internal transcribed spac..
Aspergillus flavus isolate A254_12 18S ribosomal RNA gene, partial sequence; internal transcribed spac..
Aspergillus oryzae strain P27C3A 18S ribosomal RNA gene, partial sequence; internal transcribed spacer.
Aspergillus minisclerotigenes isolate C55 18S ribosomal RNA gene, partial sequence; internal transcribed
Aspergillus oryzae strain S2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, c...
ascomycetes | 15 leaves
Aspergillus flavus isolate A251_17 18S ribosomal RNA gene, partial sequence; internal transcribed spac..
Aspergillus flavus strain ATCC 200026 18S ribosomal RNA gene, partial sequence; internal transcribed ...
Aspergillus flavus isolate YLA-65 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge...
Uncultured endophytic fungus clone R101 18S ribosomal RNA gene, partial sequence
Aspergillus flavus strain SGE22 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
Davidiella sp. FM-9G 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribos
Aspergillus oryzae strain C2-11 18S ribosomal RNA gene, partial sequence; and internal transcribed spac.
Aspergillus oryzae strain C1-7 18S ribosomal RNA gene, partial sequence; and internal transcribed spac...
Aspergillus flavus strain A-3196 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
Aspergillus oryzae strain A005 18S ribosomal RNA gene, partial sequence
Aspergillus flavus isolate RF1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.
Aspergillus oryzae isolate MD21_17 18S ribosomal RNA gene, partial sequence; internal transcribed spa..
Aspergillus flavus strain SGE34 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
Aspergillus sp. QHL15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ri...
Aspergillus sp. PC-2012 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S r.
Aspergillus flavus strain ATCC 11489 18S ribosomal RNA gene, partial sequence; internal transcribed sp.
Aspergillus flavus strain NIOCC 107 18S ribosomal RNA gene, partial sequence; internal transcribed spa..
Aspergillus oryzae isolate F5-03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
Aspergillus flavus isolate F4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus oryzae strain MQ2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...
Aspergillus oryzae genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequen...
Aspergillus oryzae strain Asor-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
kl12059
ascomycetes | 11 leaves
Aspergillus flavus isolate A353_D3 18S ribosomal RNA gene, partial sequence; internal transcribed spac.
Aspergillus oryzae strain P6B2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,.
Aspergillus flavus strain P27C3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
Aspergillus flavus strain DAOM 225949 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...
Aspergillus flavus culture-collection MUM:10.202 18S ribosomal RNA gene, partial sequence; internal tra..
Aspergillus flavus culture-collection NRRL:6412 18S ribosomal RNA gene, partial sequence; internal tran.
Aspergillus flavus isolate CZN-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
Aspergillus oryzae strain DG-C3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
Aspergillus oryzae strain DG-B3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...
Aspergillus flavus strain 11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus flavus strain MSSRFI52 18S ribosomal RNA gene, partial sequence; internal transcribed sp...
Aspergillus flavus strain wxm103 18S ribosomal RNA gene, partial sequence; internal transcribed spacer.
Aspergillus flavus isolate UOA/MCPF8374A 18S ribosomal RNA gene, partial sequence; internal transcr...
Fungal endophyte sp. LX01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
ascomycetes | 7 leaves
Aspergillus flavus strain AF01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 ...
0.0002

Aspergillus niger strain DF09002 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5

Sequence ID: gb|GU338398.1| Length: 583 Number of Matches: 2

Range 1: 15 to 564 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
984 bits(1090)	0.0	549/550(99%)	1/550(0%)	Plus/Minus

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Query  1    GAGGTCA-CCTGGAAAAATGGTTGGAAAACGTCGGCAGGCGCCGGCCAATCCTACAGAGC  59
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Sbjct  564  GAGGTCAACCTGGAAAAATGGTTGGAAAACGTCGGCAGGCGCCGGCCAATCCTACAGAGC  505

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Sbjct  504  ATGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCC  445

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Sbjct  384  ACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCG  325

Query  240  ATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGA  299
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Sbjct  324  ATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGA  265

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Sbjct  144  AAGGCGCCCCCCCGGCGGCCGACAAGCGGCGGGCCCGCCGAAGCAACAGGGTATAATAGA  85

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Query  540  CCTACGGAAA  549
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Sbjct  24   CCTACGGAAA  15
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Aspergillus niger internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Aspergillus niger strain MPVCT 158 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; a...
Aspergillus niger strain MPVCT 344 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; a...
Aspergillus niger isolate An3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, parti...
Aspergillus niger isolate A2S1_D105 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; a...
Aspergillus niger strain BE-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S r...
ascomycetes | 2 leaves
Aspergillus niger strain IHB F 1831 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, co...
Aspergillus niger ITS1, 5.8S rRNA gene and ITS2, strain MACN2
Aspergillus niger isolate EFB1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, comple...
Aspergillus awamori ITS region; from TYPE material
Aspergillus niger strain K.chilly 4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete seque...
Aspergillus niger strain ATCC 1015 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, co...
Aspergillus niger strain KAML02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, comp...
Aspergillus niger 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; a...
Aspergillus sp. 6 BRO-2013 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete s...
Aspergillus niger strain WA0000019042 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...
Uncultured fungus clone CMH451 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, com...
Aspergillus niger strain DF09002 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, ...
Aspergillus cf. tubingensis KRCF700 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, partial and complete sequence
Aspergillus tubingensis strain NJA-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge...
ascomycetes | 2 leaves
Aspergillus tubingensis partial 18S rRNA gene, 5.8S rRNA gene, partial 28S rRNA gene, internal transcribed spacer 1 (ITS1) and inter...
Uncultured fungus clone Z1-24 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribos...
Aspergillus tubingensis IBL 03117 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene,...
ascomycetes | 9 leaves
Aspergillus tubingensis strain Ed8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, ...
Aspergillus niger strain EIM-6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and ...
Aspergillus niger strain CPGM 1109 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge...
Aspergillus sp. 205 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr...
Aspergillus niger isolate South-west0093 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA...
ascomycetes | 9 leaves
Aspergillus niger genes for small subunit rRNA, ITS1, 5.8S rRNA, ITS2 and large subunit rRNA, partial and complete sequence, strain: I...
Aspergillus tubingensis strain CNU081066 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal R...
Aspergillus sp. s014 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete s...
Aspergillus tubingensis strain GX1-5E 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA g...
ascomycetes | 4 leaves
Aspergillus niger isolate South-west0062 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA...
Aspergillus tubingensis isolate South-west0056 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal...
Gliocladium cibotii isolate South-west0039 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal R...
Aspergillus sp. DCS31 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal...
Aspergillus sp. r308 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr...
lcl|62301
Aspergillus cf. tubingensis FunS29 18S ribosomal RNA gene, partial sequenc...
Aspergillus awamori 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr...
Aspergillus sp. 06 SMR-2010 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and ...
Aspergillus niger strain WM10.72 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, ...
ascomycetes | 12 leaves
Aspergillus niger strain WM10.74 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, ...
Aspergillus niger strain WM10.75 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, ...
Aspergillus niger strain WM10.78 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, ...
Aspergillus tubingensis isolate JH01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge...
Uncultured fungus clone Z1-38 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a...
ascomycetes | 16 leaves
0.001

>emb|AM262390.1| Acremonium strictum 5.8S rRNA gene, 28S rRNA gene, ITS1 and ITS2, isolate 1340
Length=504

Score = 899 bits (996), Expect = 0.0
Identities = 502/503 (99%), Gaps = 1/503 (0%)
Strand=Plus/Minus

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Sbjct  323  ATGACGCTCGGACAGGCATGCCCGCCGGAGTGCCGGCGGGCGCAATGTGCGTTCAAAGAT  264

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Query  420  CCCGGAGGCCACCGCGCGCTGAGCCCGCCGAGGGAACGTTTGGTAAGTTCACAATGGGTT  479
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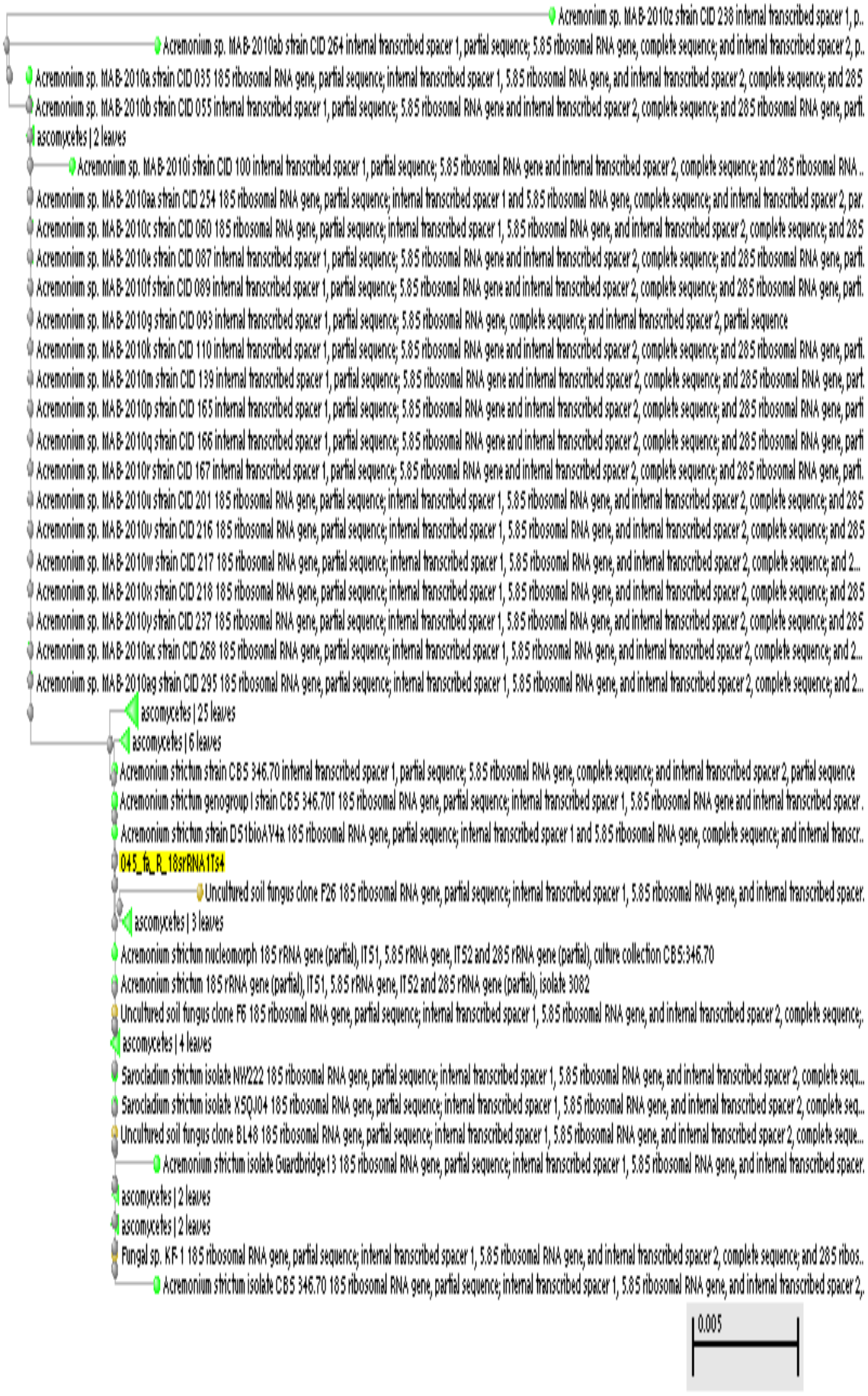
Acremonium sp. MAB-2010z strain CID 238 internal transcribed spacer 1, p..
Acremonium sp. MAB-2010ab strain CID 264 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, p..
Acremonium sp. MAB-2010a strain CID 035 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S
Acremonium sp. MAB-2010b strain CID 055 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, parti.
ascomycetes | 2 leaves
Acremonium sp. MAB-2010i strain CID 100 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA ..
Acremonium sp. MAB-2010aa strain CID 254 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, par.
Acremonium sp. MAB-2010c strain CID 060 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S
Acremonium sp. MAB-2010e strain CID 087 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, parti.
Acremonium sp. MAB-2010f strain CID 089 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, parti.
Acremonium sp. MAB-2010g strain CID 093 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Acremonium sp. MAB-2010k strain CID 110 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, parti.
Acremonium sp. MAB-2010m strain CID 139 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, part.
Acremonium sp. MAB-2010p strain CID 165 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, parti
Acremonium sp. MAB-2010q strain CID 166 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, parti
Acremonium sp. MAB-2010r strain CID 167 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, parti.
Acremonium sp. MAB-2010u strain CID 201 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S
Acremonium sp. MAB-2010v strain CID 216 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S
Acremonium sp. MAB-2010w strain CID 217 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Acremonium sp. MAB-2010x strain CID 218 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S
Acremonium sp. MAB-2010y strain CID 237 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S
Acremonium sp. MAB-2010ac strain CID 268 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Acremonium sp. MAB-2010ag strain CID 295 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
ascomycetes | 25 leaves
ascomycetes | 6 leaves
Acremonium strictum strain CBS 346.70 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Acremonium strictum genogroup I strain CBS 346.70T 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer .
Acremonium strictum strain D51bioAV4a 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcr..
045_fa_R_18srRNA1ITs4
Uncultured soil fungus clone F26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer.
ascomycetes | 3 leaves
Acremonium strictum nucleomorph 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), culture collection CBS:346.70
Acremonium strictum 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), isolate 3082
Uncultured soil fungus clone F6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence;.
ascomycetes | 4 leaves
Sarocladium strictum isolate NW222 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequ...
Sarocladium strictum isolate KSQJ04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq...
Uncultured soil fungus clone BL48 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seque...
Acremonium strictum isolate Guardbridge13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer.
ascomycetes | 2 leaves
ascomycetes | 2 leaves
Fungal sp. KF-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribos..
Acremonium strictum isolate CBS 346.70 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,
0.005

>gb|JF682635.1| Penicillium verruculosum strain PTC06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Length=550

Score = 901 bits (998), Expect = 0.0
Identities = 510/516 (99%), Gaps = 1/516 (0%)
Strand=Plus/Plus

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Sbjct  75   CCGGGGGACGTTCGTCCCCGGGCCCGCGCCCGCCGAAGCGCTCTGTGAACCCTGATGAAG  134

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Sbjct  135  ATGGGCTGTCTGAGTACTATGAAAATTGTCAAAACTTTCAACAATGGATCTCTTGGTTCC  194

Query  181  GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAA  240
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Sbjct  195  GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAA  254

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Sbjct  255  TCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGA  314

Query  301  GCGTCATTTCTGCCCTCAAGCACGGCTTGTGTGTTGGGTGTGGTccccccGGGGACCTG  360
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Sbjct  315  GCGTCATTTCTGCCCTCAAGCACGGCTTGTGTGTTGGGTGTGGT-CCCCCCGGGGACCTG  373

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Sbjct  374  CCCGAAAGGCAGCGGCGACGTCCGTCTGGTCCTCGAGCGTATGGGGCTCTGTCACTCGCT  433

Query  421  CGGGAAGGACCTGCGGAGGTTGGTCACCACCATATTTTACCACGGTTGACCTCGGATCAG  480
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Sbjct  434  CGGGAAGGACCTGCGGGGGTTGGTCACCACCATATTTTACCACGGTTGACCTCGGATCAG  493

Query  481  GTAGGAGTTACCCGCTGAACTTAACCATATCAATAA  516
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Sbjct  494  GTAGGAGTTACCCGCTGAACTTAAGCATATCAATAA  529
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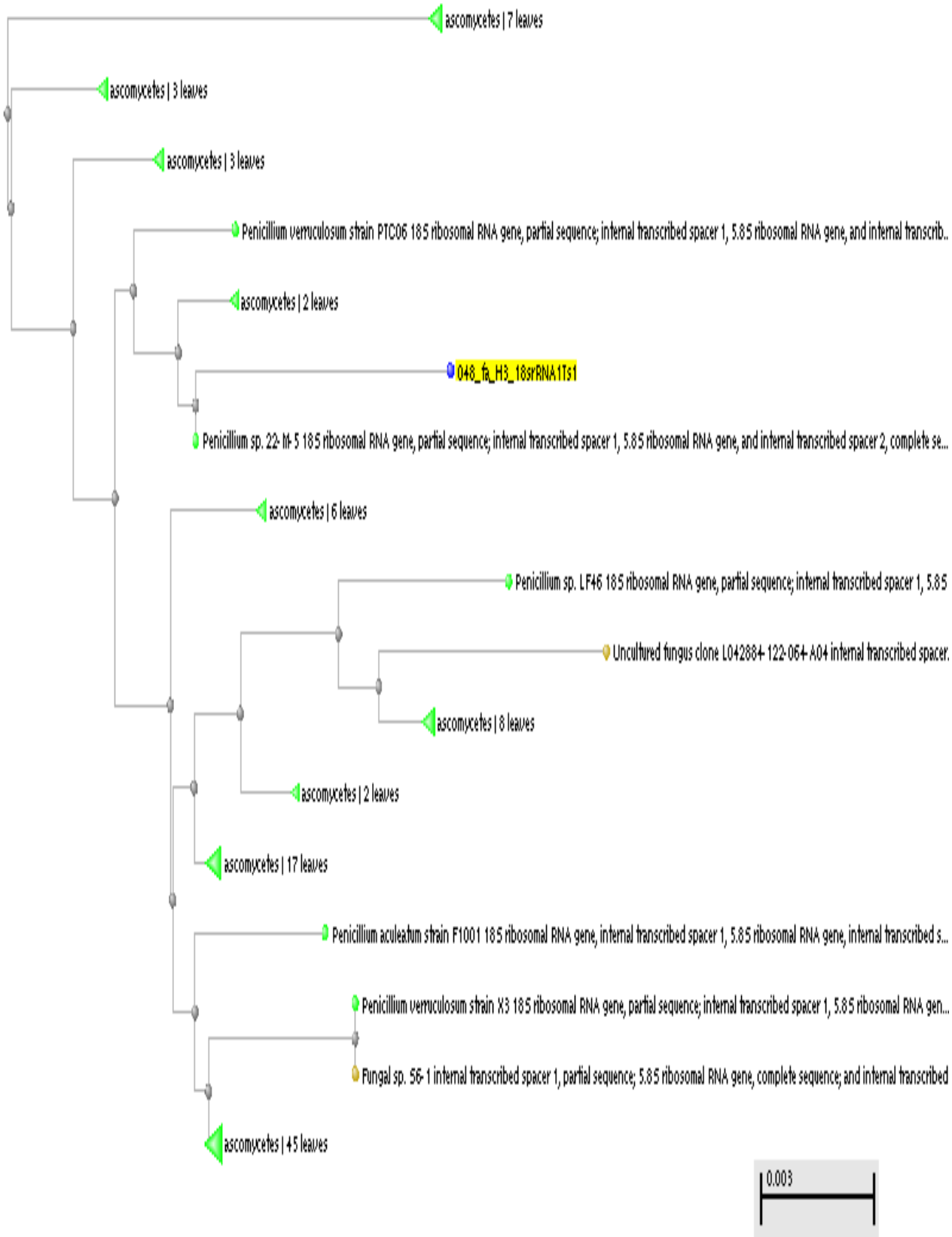
ascomycetes | 7 leaves
ascomycetes | 3 leaves
ascomycetes | 3 leaves
Penicillium verruculosum strain PTC06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcrib..
ascomycetes | 2 leaves
048_fa_H3_18srRNA1Ts1
Penicillium sp. 22-M-5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete se...
ascomycetes | 6 leaves
Penicillium sp. LF46 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S
Uncultured fungus clone L042884-122-064-A04 internal transcribed spacer.
ascomycetes | 8 leaves
ascomycetes | 2 leaves
ascomycetes | 17 leaves
Penicillium aculeatum strain F1001 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed s...
Penicillium verruculosum strain X3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gen...
Fungal sp. S6-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed
ascomycetes | 45 leaves
0.003

>gb|JQ912672.1| Mucor hiemalis 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Length=799

Score = 623 bits (337), Expect = 3e-175
Identities = 353/361 (98%), Gaps = 0/361 (0%)
Strand=Plus/Minus

```
Query  1    TAATACTAGAGCATTCCTTTATATTaaaaaaaTGTTCAGGCAGAAAGAACAATGGTTCAG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  688  TAATACTAGAGCATTCCTTTATATTAAAAAAATGTTCAGGCAGAAAGAACAATAGTTCAG  629

Query  61   GCCTAATAAGTTTAAAGAATTCAAGCAAGTCGAAATTCTCAGTTCCATTCACAACAAAAT  120
            |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  628  GCCTAATAAGTTTAAAGAATTCAAACAAGTCGAAATTCTCAGTTCCATTCACAACAAAAT  569

Query  121  TATGAATGTGGGGTGTTTTTGATACTGAAACAGGCGTGCTCTATGGAATACCATTGAGCG  180
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  568  TATGAATGTGGGGTGTTTTTGATACTGAAACAGGCGTGCTCAATGGAATACCATTGAGCG  509

Query  181  CTAGTTGCGTTCAAAGACTCGATGATTCACTGAATATGCAATTCACACTAGTTATCGCAC  240
            | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  508  CAAGTTGCGTTCAAAGACTCGATGATTCACTGAATATGCAATTCACACTAGTTATCGCAC  449

Query  241  TTTGCTACGTTCTTCATCGATGCGAGAACCCAGAGATCCGTTGTTAAAAGTTGTTTTATA  300
            |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  448  TTTGCTACGTTCTTCATCGATGCGAGAACCAAGAGATCCGTTGTTAAAAGTTGTTTTATA  389

Query  301  AGTTTTTTACGCTCATGTTACAATAATAATACTGAATTCTTTTGGTaaaaaaaTAATAGG  360
            |||||||||||||| ||||||||||||||||||||||||||||||| || |||||||||||
Sbjct  388  AGTTTTTTACGCTTATGTTACAATAATAATACTGAATTCTTTTGGTAAATAATTAATAGG  329

Query  361  A  361
            |
Sbjct  328  A  328
```


fungi | 11 leaves
fungi | 2 leaves
fungi | 33 leaves
Mucor sp. FJ09 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
fungi | 2 leaves
Mucor sp. BEA-2010 isolate A1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial seque...
Mucor hiemalis culture-collection F5U<DEU>:10221 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Mucor sp. BEA-2010 isolate B2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene,..
Mucor hiemalis strain 2511A internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Mucor hiemalis culture-collection F5U<DEU>:10222 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA ...
Mucor hiemalis 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Mucor sp. BEA-2010 isolate B3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, p..
Mucor hiemalis culture-collection F5U<DEU>:9871 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial se...
Mucor hiemalis culture-collection F5U<DEU>:9902 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S rib...
Mucor hiemalis culture-collection F5U<DEU>:9903 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial se...
Mucor hiemalis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), strain CBS 201.65
Mucor hiemalis strain F5U6530 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, pa...
Mucor hiemalis f. hiemalis strain F5U 6179 clone 1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribos...
lcl|63925
Mucor hiemalis f. hiemalis strain CBS 201.65 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal ...
fungi | 10 leaves
fungi | 27 leaves
0.005

- **phylogenetic analysis of fungi and bacteria related to alginate**

Bacillus licheniformis strain LZBL-11 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|JX847117.1| Length: 1083 Number of Matches: 1

Range 1: 232 to 1026 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1364 bits(1512)	0.0	781/796(98%)	1/796(0%)	Plus/Minus

```
Query  32    TACCTCTGCCAATTACGGTGGTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGC  91
             ||||||  | | ||  |||| ||||||||||||||||||||||||||||||||||||||
Sbjct  1026  TACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGC  967

Query  92    CCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCACGC  151
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  966   CCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCACGC  907

Query  152   AGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGATTGGCTTAGCCTCG  211
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  906   AGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGATTGGCTTAGCCTCG  847

Query  212   CGGCTTCGCTGCCCTTTGTTCTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGG  271
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  846   CGGCTTCGCTGCCCTTTGTTCTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGG  787

Query  272   CATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAG  331
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  786   CATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAG  727

Query  332   TGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCA  391
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  726   TGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCA  667

Query  392   ACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGAAGGG  451
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  666   ACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGAAGGG  607

Query  452   GAAGCCCTATCTCTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGC  511
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  606   GAAGCCCTATCTCTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGC  547

Query  512   TTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTC  571
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  546   TTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTC  487

Query  572   AGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTTGCTGCAGCACTAAAAGG  631
             |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  486   AGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTTGCTGCAGCACT-AAAGG  428

Query  632   GCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAAGGTATCTAA  691
             |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  427   GCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAA  368

Query  692   TCCTGTTCGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAAACCAGAGAGTCGCCTT  751
             |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  367   TCCTGTTCGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTT  308

Query  752   CGCCCCTGGTGTTCCTCCACATCTCTACACATTTCACCGCTACACGGGGAATTCCCTCCC  811
             |||| |||||||||||||||||||| |||||||||||||||| ||||||||| |||
Sbjct  307   CGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCCCCCC  248

Query  812   CTTCTTCTGCACTCAA  827
             | ||||||||||||||
Sbjct  247   CCTCTTCTGCACTCAA  232
```


044 fa MB1 16SR
firmicutes | 100 leaves
0.0006

Planococcus sp. Or1 16S ribosomal RNA gene, complete sequence

Sequence ID: gb|JF742665.1| Length: 1555 Number of Matches: 1

Range 1: 44 to 913 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1535 bits(1702)	0.0	864/871(99%)	1/871(0%)	Plus/Plus

```
Query  1    GCGTGCCTAATACGTGGGAATTCGAGGGGAACCAGAGGAGCTTGCTCCTTCTGGTTTAGC  60
            ||||||||||||| ||  || ||||| ||||||||||||||||||||||||||||||||||
Sbjct  44   GCGTGCCTAATACATGC-AAGTCGAGCGGAACCAGAGGAGCTTGCTCCTTCTGGTTTAGC  102

Query  61   GGCGGACGGGTGAGTAACACGTGGGCAACCTGCCCTGCAGATCGGGATAACTCCGGGAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  103  GGCGGACGGGTGAGTAACACGTGGGCAACCTGCCCTGCAGATCGGGATAACTCCGGGAAA  162

Query  121  CCGGTGCTAATACCGAATAGTTTGCGGCCTCTCCTGAGGCTGCACGGAAAGACGGTTTCG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163  CCGGTGCTAATACCGAATAGTTTGCGGCCTCTCCTGAGGCTGCACGGAAAGACGGTTTCG  222

Query  181  GCTGTCACTGCAGGATGGGCCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCCTACCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  223  GCTGTCACTGCAGGATGGGCCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCCTACCA  282

Query  241  AGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  283  AGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  342

Query  301  CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  343  CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  402

Query  361  GCAACGCCGCGTGAGTGACGAAGGTTTTCGGATCGTAAAACTCTGTTGTGAGGGAAGAAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403  GCAACGCCGCGTGAGTGACGAAGGTTTTCGGATCGTAAAACTCTGTTGTGAGGGAAGAAC  462

Query  421  AAGTACCAACTAACTACTGGTACCTTGACGGTACCTCACCAGAAAGCCACGGCTAACTAC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  463  AAGTACCAACTAACTACTGGTACCTTGACGGTACCTCACCAGAAAGCCACGGCTAACTAC  522

Query  481  GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  523  GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAA  582

Query  541  GCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC  600
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  583  GCGCGCGCAGGCGGTTCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC  642

Query  601  ATTGGAAACTGGAGGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGA  660
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  643  ATTGGAAACTGGAGAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGA  702

Query  661  AATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGAC  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  703  AATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGAC  762

Query  721  GCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  763  GCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA  822

Query  781  AACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAA  840
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  823  AACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAA  882

Query  841  GCACTCCGCCTGGGGAGTACGGCCGCAAGGC  871
            |||||||||||||||||||||||||||||||
Sbjct  883  GCACTCCGCCTGGGGAGTACGGCCGCAAGGC  913
```


Planococcus antarcticus strain HWG-A5 16S ribosomal RNA gene, partial sequence
Uncultured Planococcaceae bacterium clone hsc489 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
firmicutes | 2 leaves
Planococcus sp. MTCC 8491 16S ribosomal RNA gene, partial sequence
Planococcus maritimus strain KMM 3738 16S ribosomal RNA gene, complete sequence
Planococcus maritimus strain IT-9 16S ribosomal RNA, partial sequence
Planococcus sp. MMRF526 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone Q31007 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
bacteria | 5 leaves
Uncultured bacterium clone D30930 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D21029 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D21026 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D21021 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D10930 16S ribosomal RNA gene, partial sequence
Planococcus sp. Or1 16S ribosomal RNA gene, complete sequence
Planococcus sp. NP19 16S ribosomal RNA gene, partial sequence
Planococcus sp. Nj-73 16S rRNA gene, strain Nj-73
Planococcus sp. p16X 16S rRNA gene
Planococcus sp. SOZ2-2141 16S ribosomal RNA gene, partial sequence
bacteria | 4 leaves
034 fa MB2 16SF
firmicutes | 3 leaves
firmicutes | 3 leaves
firmicutes | 3 leaves
Planococcus donghaensis strain L10.15 16S ribosomal RNA gene, partial sequence
Planococcus sp. SOD-1302 16S ribosomal RNA gene, partial sequence
firmicutes | 3 leaves
bacteria | 3 leaves
Planomicrobium okeanokoites strain HWG-A10 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1105200082402 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
bacteria | 16 leaves
bacteria | 2 leaves
bacteria | 19 leaves
Planococcus sp. TP-Snow-C20 16S ribosomal RNA gene, partial sequence
bacteria | 3 leaves
bacteria | 6 leaves
0.001

Planococcus donghaensis strain JH1 16S ribosomal RNA, partial sequence

Sequence ID: ref|NR_044073.1| Length: 1452 Number of Matches: 1

See 1 more title(s)

Range 1: 16 to 895 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1494 bits(1656)	0.0	863/881(98%)	4/881(0%)	Plus/Plus

```
Query  1    CGTGCCTAATACATGCAAGTCGAGGGGAACCAGAGGAGCTTGCTCCTTCTGGTTTAGCGG  60
            ||| |||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  16   CGTNCCTAATACATGCAAGTCGAGCGGAACCAGAGGAGCTTGCTCCTTCTGGTTTAGCGG  75

Query  61   CGGACGGGTGAGTAACACGTGGGCAACCTGCCCTGCAGATCGGGATAACTCCGGGAAACC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  76   CGGACGGGTGAGTAACACGTGGGCAACCTGCCCTGCAGATCGGGATAACTCCGGGAAACC  135

Query  121  GGTGCTAATACCGAATAGTTTGCGGCCTCTCCTGAGGCTGCACGGAAAGACGGTTTCGGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  136  GGTGCTAATACCGAATAGTTTGCGGCCTCTCCTGAGGCTGCACGGAAAGACGGTTTCGGC  195

Query  181  TGTCACTGCAGGATGGGCCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCCTACCAAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  196  TGTCACTGCAGGATGGGCCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCCTACCAAG  255

Query  241  GCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  256  GCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC  315

Query  301  AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  316  AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC  375

Query  361  AACGCCGCGTGAGTGACGAAGGTTTTCGGATCGTAAAACTCTGTTGTGAGGGAAGAACAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  376  AACGCCGCGTGAGTGACGAAGGTTTTCGGATCGTAAAACTCTGTTGTGAGGGAAGAACAA  435

Query  421  GTACCAACTAACTACTGGTACCTTGACGGTACCTCACCAGAAAGCCACGGCTAACTACGT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  436  GTACCAACTAACTACTGGTACCTTGACGGTACCTCACCAGAAAGCCACGGCTAACTACGT  495

Query  481  GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  496  GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGC  555

Query  541  GCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556  GCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCAT  615

Query  601  TGGAAACTGGAGGACTTGAGTGCAGAAAAGGAAAGTGGAATTCCATGTGTAGCGGTGAAA  660
            |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  616  TGGAAACTGGAGGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAA  675

Query  661  TGCGTAAAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACCC  720
            |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  676  TGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGC  735

Query  721  TGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAAATACCCTGGTAGTCCACGCCGT-AA  779
            |||||||||||||||||||||||||||||||||| |||||||||||||||||||| ||
Sbjct  736  TGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA  795

Query  780  CGATGAGTGCT-AGTGTTAAGGGGGTTTcc-ccccTTAATGCTGCACCTAACCCATTAA  837
            |||||||||||| |||||| |||||||||||| |||| ||| ||||||| ||||| ||||||
Sbjct  796  CGATGAGTGCTAAGTGTT-AGGGGGTTTCCGCCCCTTTAGTGCTGCAGCTAACGCATTAA  854

Query  838  CCACTCCCCCTGGGGAATACGGCCCCAAGGCTGAAACTCAA  878
              |||||| |||||||| ||||||| ||||||||||||||||
Sbjct  855  GCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAA  895
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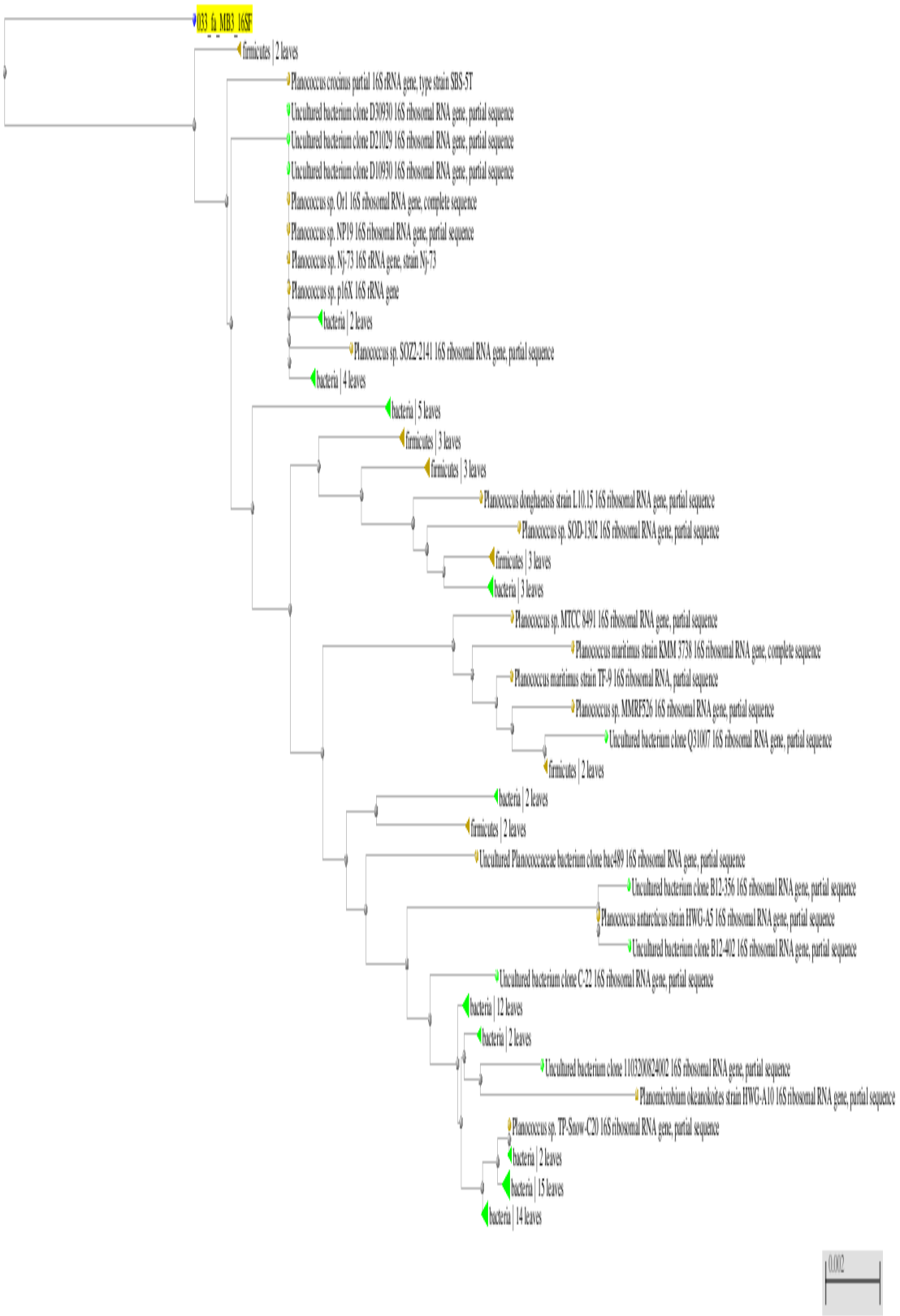
033_fa_MB3_16SF
firmicutes | 2 leaves
Planococcus crocinus partial 16S rRNA gene, type strain SBS-5T
Uncultured bacterium clone D30930 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D21029 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D10930 16S ribosomal RNA gene, partial sequence
Planococcus sp. Or1 16S ribosomal RNA gene, complete sequence
Planococcus sp. NP19 16S ribosomal RNA gene, partial sequence
Planococcus sp. Ni-73 16S rRNA gene, strain Ni-73
Planococcus sp. p16X 16S rRNA gene
bacteria | 2 leaves
Planococcus sp. SOZ2-2141 16S ribosomal RNA gene, partial sequence
bacteria | 4 leaves
bacteria | 5 leaves
firmicutes | 3 leaves
firmicutes | 3 leaves
Planococcus donghaensis strain L10.15 16S ribosomal RNA gene, partial sequence
Planococcus sp. SOD-1302 16S ribosomal RNA gene, partial sequence
firmicutes | 3 leaves
bacteria | 3 leaves
Planococcus sp. MTCC 8491 16S ribosomal RNA gene, partial sequence
Planococcus maritimus strain KMM 3738 16S ribosomal RNA gene, complete sequence
Planococcus maritimus strain TF-9 16S ribosomal RNA, partial sequence
Planococcus sp. MMRF526 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone Q31007 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
bacteria | 2 leaves
firmicutes | 2 leaves
Uncultured Planococcaceae bacterium clone bac489 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone B12-356 16S ribosomal RNA gene, partial sequence
Planococcus antarcticus strain HWG-A5 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone B12-402 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C-22 16S ribosomal RNA gene, partial sequence
bacteria | 12 leaves
bacteria | 2 leaves
Uncultured bacterium clone 1103200824002 16S ribosomal RNA gene, partial sequence
Planomicrobium okeanokoites strain HWG-A10 16S ribosomal RNA gene, partial sequence
Planococcus sp. TP-Snow-C20 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
bacteria | 15 leaves
bacteria | 14 leaves
0.002

Planococcus sp. SS1.3 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KC160837.1| Length: 1511 Number of Matches: 1

Range 1: 575 to 1473 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1561 bits(1730)	0.0	888/901(99%)	2/901(0%)	Plus/Minus

```
Query  1     ACCTTCGGCGGCTGGGCTCCACAAGGGTTACCTCACCGACTTCGGGTGTTACAAACTCTC  60
             ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1473  ACCTTCGGCGGCTGG-CTCCACAAGGGTTACCTCACCGACTTCGGGTGTTACAAACTCTC  1415

Query  61    GTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATGCTGATCC  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1414  GTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATGCTGATCC  1355

Query  121   ACGATTACTAGCGATTCCGGCTTCATGCAGGCGAGTTGCAGCCTACAATCCGAACTGAGA  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1354  ACGATTACTAGCGATTCCGGCTTCATGCAGGCGAGTTGCAGCCTACAATCCGAACTGAGA  1295

Query  181   ACGGTTTTATGGGATTGGCTCCCCCTCGCGGGTTGGCAACCCTTTGTACCGTCCATTGTA  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1294  ACGGTTTTATGGGATTGGCTCCCCCTCGCGGGTTGGCAACCCTTTGTACCGTCCATTGTA  1235

Query  241   GCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1234  GCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTC  1175

Query  301   CGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAG  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1174  CGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAG  1115

Query  361   GGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1114  GGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG  1055

Query  421   CACCACCTGTCACCATTGTCCCCGAAGGGAAAAGTGTATCTCTACACCGGGCAATGGGAT  480
             ||||||||||||||| ||||||||||||||||||||||||||||||| ||||||
Sbjct  1054  CACCACCTGTCACCACTGTCCCCGAAGGGAAAAGTGTATCTCTACACCGGGCAGTGGGAT  995

Query  481   GTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTG  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  994   GTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTG  935

Query  541   TGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGANT  600
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  934   TGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGT  875

Query  601   GCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCG  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  874   GCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCG  815

Query  661   TTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCA  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  814   TTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCA  755

Query  721   GCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCA  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  754   GCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCA  695

Query  781   TTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCCTCCAGTTTCCA  840
             |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  694   TTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTCTCCAGTTTCCA  635

Query  841   ATGACCCTCCACGGTTGAACCGGGGGCTTTCCCATCAAAATTAAAAGGACCGCCTGCCCG  900
             |||||||||||||||||| ||| |||||||| ||||| | || |||| |||||||||| ||
Sbjct  634   ATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT-AAAGAACCGCCTGCGCG  576

Query  901   C  901
             |
Sbjct  575   C  575
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041 fa MB4 16SR
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: IC-24
Planococcus donghaensis strain L10.15 16S ribosomal RNA gene, partial sequence
Bacterium BW15W14 16S ribosomal RNA gene, partial sequence
Planococcus sp. SOD-1302 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone My46-484 16S ribosomal RNA gene, partial sequence
Planococcus sp. BJ-1 16S ribosomal RNA gene, partial sequence
Planococcus sp. SS1.3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D10930 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
firmicutes | 6 leaves
bacteria | 4 leaves
Planococcus sp. SS1.4 16S ribosomal RNA gene, partial sequence
Planococcus sp. SS9.30 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D21029 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
Planococcus crocinus partial 16S rRNA gene, type strain SBS-5T
firmicutes | 3 leaves
firmicutes | 3 leaves
bacteria | 2 leaves
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 12TCLN305
Uncultured bacterium clone SINI867 16S ribosomal RNA gene, partial sequence
Uncultured Planococcus sp. clone 24 16S ribosomal RNA gene, partial sequence
Planococcus antarcticus strain HWG-A5 16S ribosomal RNA gene, partial sequence
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 12TCLN306
Uncultured bacterium clone B12-356 16S ribosomal RNA gene, partial sequence
Planomicrobium sp. MDT3-16 16S ribosomal RNA gene, partial sequence
firmicutes | 8 leaves
Uncultured Planococcus sp. clone ASC190 16S ribosomal RNA gene, partial sequence
Uncultured bacterium partial 16S rRNA gene, clone FB02G06
Planococcus psychrotoleratus isolate S3-21 16S ribosomal RNA gene, partial sequence
Planomicrobium glaciei partial 16S rRNA gene, strain CLPB29
bacteria | 2 leaves
Glacial ice bacterium G50-TS4 16S ribosomal RNA gene, partial sequence
firmicutes | 3 leaves
Uncultured bacterium clone TE-2-E7 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone TE-3-B11 16S ribosomal RNA gene, partial sequence
Planomicrobium sp. Hh17 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone TE-2-B5 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1103200828492 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1103200828410 16S ribosomal RNA gene, partial sequence
Uncultured rumen bacterium clone BRC25 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1103200828802 16S ribosomal RNA gene, partial sequence
bacteria | 3 leaves
bacteria | 3 leaves
bacteria | 9 leaves
bacteria | 3 leaves
bacteria | 3 leaves
bacteria | 6 leaves
Planococcus sp. C2-4c-3 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
Uncultured bacterium clone 1103200831930 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1103200828658 16S ribosomal RNA gene, partial sequence
Planococcus sp. TP-Snow-C20 16S ribosomal RNA gene, partial sequence
0.002

Bacillus stratosphericus strain MCCC 1A04568 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|JX680095.1| Length: 1513 Number of Matches: 1

Range 1: 897 to 1459 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
796 bits(882)	0.0	521/569(92%)	14/569(2%)	Plus/Minus

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Query  18    GGCTCGATAAAGGTTACCTCACCGACTTCGGGTGTTGCAAACTCTCGTGGTGTGACGGGC  77
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Sbjct  1459  GGCTCCATAAAGGTTACCTCACCGACTTCGGGTGTTGCAAACTCTCGTGGTGTGACGGGC  1400

Query  78    GGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGA  137
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399  GGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGA  1340

Query  138   TTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGA  197
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1339  TTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGA  1280

Query  198   TTGGCTAAACCTTGCGGTCTCGCAGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCC  257
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1279  TTGGCTAAACCTTGCGGTCTCGCAGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCC  1220

Query  258   CAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGG  317
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1219  CAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGG  1160

Query  318   CAGTCACCTTATAGTGCCCAACTGAATGCTGGCAACTAACATCAAGGGTTGCGCTCGTTG  377
             |||||||||| |||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  1159  CAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTG  1100

Query  378   C---ACTTATTACACCATCTTCTCACGACGCAGACTGAAAACATGCATGCCCTATCTGTC  434
             |   |||||  || ||| | ||||||||| |   ||||  |||  ||||| | | |||||
Sbjct  1099  CGGGACTTA--AC-CCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTC  1043

Query  435   TTTCCGTCCAAGAAAGAAAAG---TATCACTACTCCGGTTG--ATGGAATGTCAAGACAT  489
               || ||||  ||| | ||||   |||| |||    |||||  |  | ||||||||| |
Sbjct  1042  ACTCTGTCCCCGAAGGGAAAGCCCTATCTCTA---GGGTTGTCAGAGGATGTCAAGACCT  986

Query  490   GGTAAGGTTCTTCTCGTTGCTTCTAATTAAACCACNTGCTCCACCGCTTGTGCGGGCCCC  549
             ||||||||||||| ||||||||| |||||||||| |||||||||||||||||||||||||
Sbjct  985   GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC  926

Query  550   CGTCAATTCCTTTGATTTTCAGCCTTGCG  578
             ||||||||||||||| |||||| ||||||
Sbjct  925   CGTCAATTCCTTTGAGTTTCAGTCTTGCG  897
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048_fa_MB5_16SR
Bacillus pumilus strain BAB-244 16S ribosomal RNA gene, partial sequence
Bacillus sp. NJ-82 partial 16S rRNA gene, strain NJ-82
Bacillus sp. 4125 16S ribosomal RNA gene, partial sequence
Bacillus sp. 3127 16S ribosomal RNA gene, partial sequence
Bacillus sp. 5107 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1-42 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain T246 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR24 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR25 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR27 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR28 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR36 16S ribosomal RNA gene, partial sequence
Bacillus sp. Dca21 16S ribosomal RNA gene, partial sequence
Bacillus sp. Dca24 16S ribosomal RNA gene, partial sequence
Bacillus sp. H69 16S ribosomal RNA gene, partial sequence
Bacillus sp. S48 16S ribosomal RNA gene, partial sequence
Bacillus sp. S90 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain DF 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain EkA2-2 16S ribosomal RNA gene, partial sequence
Bacillus sp. KtRA5-106 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESR21 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain KtRA5-79 16S ribosomal RNA gene, partial sequence
Bacillus sp. KtTA4-17 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain KtMA2-6 16S ribosomal RNA gene, partial sequence
Bacillus sp. KtMA2-7 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1613 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain IARI-THW-8 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IARI-JR-46 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GC43 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GC51 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain MS42 16S ribosomal RNA gene, partial sequence
Bacillus sp. EkMoC1-M1 16S ribosomal RNA gene, partial sequence
Uncultured Bacillus sp. clone AS6-G 16S ribosomal RNA gene, partial sequence
Bacillus sp. AHBR17 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IHB B 1045 16S ribosomal RNA gene, partial sequence
Bacterium R-3 16S ribosomal RNA gene, partial sequence
Bacillus sp. C63 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZB12 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain 126 (BC21M) 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain BAB-214 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-2829 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1830 16S ribosomal RNA gene, partial sequence
Bacillus sp. Gr-11 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00008 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02227 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A04568 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain T86 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain T75 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain Nsic-2 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain B3 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain V1 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C158 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C72 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain B12 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain B5 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-7 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone SV-10 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain B65 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain Z4S2 16S ribosomal RNA gene, partial sequence
Bacillus sp. JS3 16S ribosomal RNA gene, partial sequence
Bacillus sp. DMB1 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain MD 02 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone CDX31 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain OC4 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain M7 16S ribosomal RNA gene, partial sequence
Bacillus sp. VITPGMB partial 16S rRNA gene, isolate PGMB1
Bacillus sp. XJ9DNR2 gene for 16S rRNA, partial sequence
Bacillus sp. XJ9DR2 gene for 16S rRNA, partial sequence
Bacillus sp. XJ9DR1 gene for 16S rRNA, partial sequence
Bacillus pumilus 16S ribosomal RNA gene, partial sequence
Bacillus sp. 5120 16S ribosomal RNA gene, partial sequence
Bacillus sp. 5101 16S ribosomal RNA gene, partial sequence
Bacillus sp. 5096 16S ribosomal RNA gene, partial sequence
Bacterium BW3SW13 16S ribosomal RNA gene, partial sequence
firmicutes | 24 leaves
Uncultured bacterium clone P4 16S ribosomal RNA gene, partial sequence
0.005

Planococcus psychrotoleratus 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|AF324659.1|AF324659 Length: 1502 Number of Matches: 1

See 1 more title(s)

Range 1: 569 to 1456 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1532 bits(1698)	0.0	876/890(98%)	3/890(0%)	Plus/Minus

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Query  1     CCCACCTTCGGCGGCTGGGCTCCACAAGGGTTACCTCACCGACTTCGGGTGTTACAAACT  60
             |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  1456  CCCACCTTCGGCGGCTGG-CTCCACAAGGGTTACCTCACCGACTTCGGGTGTTACAAACT  1398

Query  61    CTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATGCTGA  120
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Sbjct  1397  CTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATGCTGA  1338

Query  121   TCCACGATTACTAGCGATTCCGGCTTCATGCAGGCGAGTTGCAGCCTACAATCCGAACTG  180
             |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  1337  TCCACGATTACTAGCGATTCCGGCTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAACTG  1278

Query  181   AGAACGGTTTTATGGGATTGGCTCCCCCTCGCGGGTTGGCAACCCTTTGTACCGTCCATT  240
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Sbjct  1277  AGAACGGTTTTCTGGGATTGGCTCCCCCTCGCGGGTTGGCAGCCCTTTGTACCGTCCATT  1218

Query  241   GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTC  300
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Sbjct  1217  GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTC  1158

Query  301   CTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATC  360
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Sbjct  1157  CTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATC  1098

Query  361   AAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACC  420
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Sbjct  1097  AAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACC  1038

Query  421   ATGCACCACCTGTCACCATTGTCCCCGAAGGGAAAAGTGTATCTCTACACCGGGCAATGG  480
             |||||||||||||||||  |||||||||||||||| | ||||||||||||||||||  ||
Sbjct  1037  ATGCACCACCTGTCACCGCTGTCCCCGAAGGGAAAGGCGTATCTCTACACCGGGCAGCGG  978

Query  481   GATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGC  540
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Sbjct  977   GATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGC  918

Query  541   TTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGG  600
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Sbjct  917   TTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGG  858

Query  601   AGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCA  660
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Sbjct  857   AGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCA  798

Query  661   TCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCC  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  797   TCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCC  738

Query  721   TCAGCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTAC  780
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Sbjct  737   TCAGCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTAC  678

Query  781   GCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCNAGTCCTCCAGTTT  840
             |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  677   GCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCCTCCAGTTT  618

Query  841   CCAATGACCCTCCACGGGTTGAGCCG-GGGCTTTCACATCAAACTTAAAG  889
             ||||||||||||||| |||||||||| ||||||||||||||| ||||||||
Sbjct  617   CCAATGACCCTCCAC-GGTTGAGCCGTGGGCTTTCACATCAGACTTAAAG  569
```

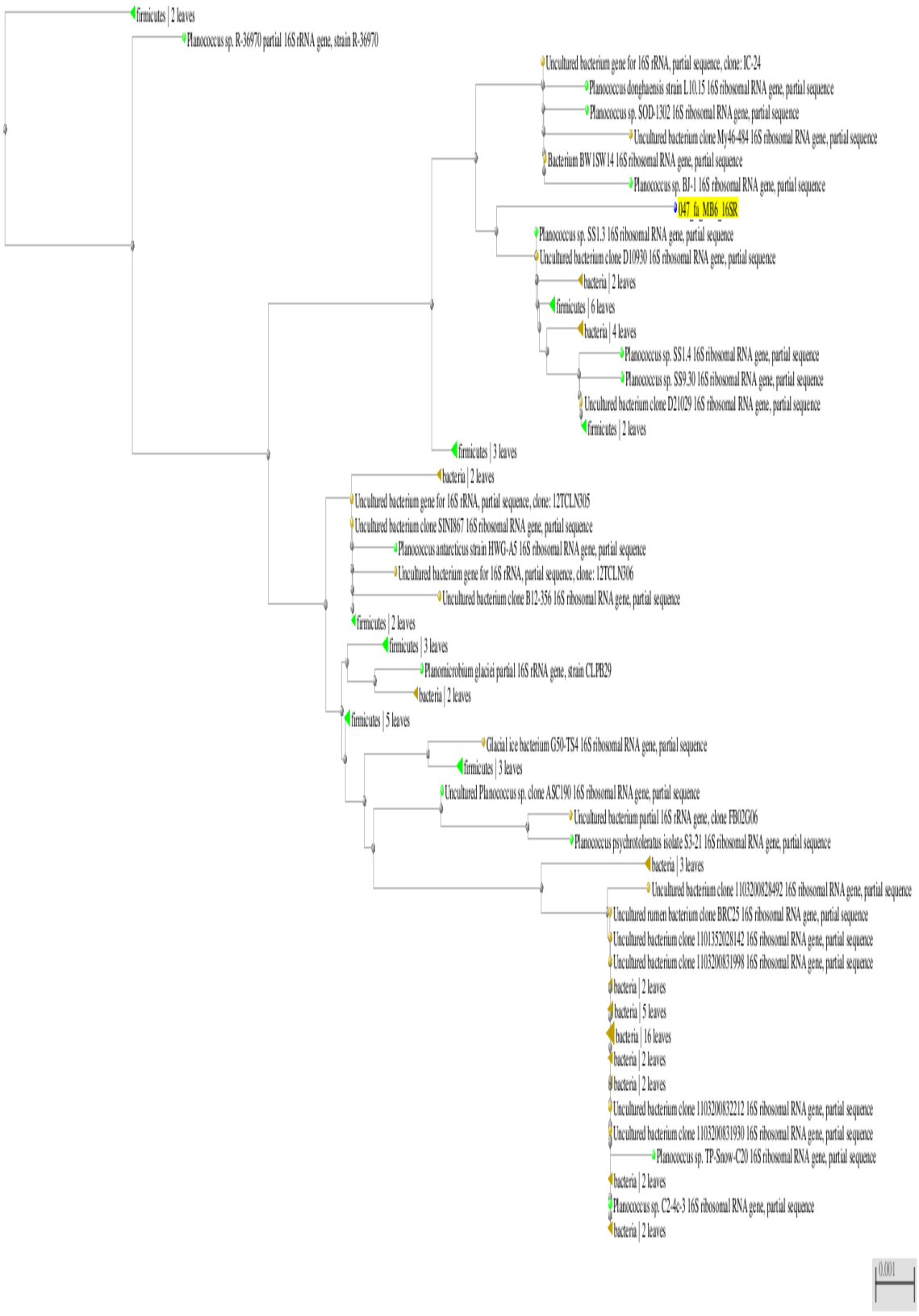
firmicutes | 2 leaves
Planococcus sp. R-36970 partial 16S rRNA gene, strain R-36970
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: IC-24
Planococcus donghaensis strain L10.15 16S ribosomal RNA gene, partial sequence
Planococcus sp. SOD-1302 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone My46-484 16S ribosomal RNA gene, partial sequence
Bacterium BW1SW14 16S ribosomal RNA gene, partial sequence
Planococcus sp. BJ-1 16S ribosomal RNA gene, partial sequence
047_fa_MB6_16SR
Planococcus sp. SS1.3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D10930 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
firmicutes | 6 leaves
bacteria | 4 leaves
Planococcus sp. SS1.4 16S ribosomal RNA gene, partial sequence
Planococcus sp. SS9.30 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D21029 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
firmicutes | 3 leaves
bacteria | 2 leaves
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 12TCLN305
Uncultured bacterium clone SINI867 16S ribosomal RNA gene, partial sequence
Planococcus antarcticus strain HWG-A5 16S ribosomal RNA gene, partial sequence
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 12TCLN306
Uncultured bacterium clone B12-356 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
firmicutes | 3 leaves
Planomicrobium glaciei partial 16S rRNA gene, strain CLPB29
bacteria | 2 leaves
firmicutes | 5 leaves
Glacial ice bacterium G50-TS4 16S ribosomal RNA gene, partial sequence
firmicutes | 3 leaves
Uncultured Planococcus sp. clone ASC190 16S ribosomal RNA gene, partial sequence
Uncultured bacterium partial 16S rRNA gene, clone FB02G06
Planococcus psychrotoleratus isolate S3-21 16S ribosomal RNA gene, partial sequence
bacteria | 3 leaves
Uncultured bacterium clone 1103200828492 16S ribosomal RNA gene, partial sequence
Uncultured rumen bacterium clone BRC25 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1101352028142 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1103200831998 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
bacteria | 5 leaves
bacteria | 16 leaves
bacteria | 2 leaves
bacteria | 2 leaves
Uncultured bacterium clone 1103200832212 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1103200831930 16S ribosomal RNA gene, partial sequence
Planococcus sp. TP-Snow-C20 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
Planococcus sp. C2-4c-3 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
0.001

Planococcus crocinus partial 16S rRNA gene, type strain SBS-5T

Sequence ID: emb|AM711973.1| Length: 1436 Number of Matches: 1

Range 1: 822 to 1406 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
893 bits(990)	0.0	555/587(95%)	6/587(1%)	Plus/Minus

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Query  35    TCTCCGACTGTCGAGTATTTCAAGCTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCG  94
             || |||||| ||| || || ||| ||||||||||||||||||||||||||||||||||||
Sbjct  1406  TCACCGACT-TCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCG  1348

Query  95    GGAACGTATTCACCGTGGCATGCTGATCCACGATTACTAGCGATTCCGGCTTCATGCAGG  154
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1347  GGAACGTATTCACCGTGGCATGCTGATCCACGATTACTAGCGATTCCGGCTTCATGCAGG  1288

Query  155   CGAGTTGCAGCCTACAATCCGAACTGAGAACGGTTTTATGGGATTGGCTCCCCCTCGCGG  214
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1287  CGAGTTGCAGCCTACAATCCGAACTGAGAACGGTTTTATGGGATTGGCTCCCCCTCGCGG  1228

Query  215   GTTGGCAACCCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCAT  274
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1227  GTTGGCAACCCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCAT  1168

Query  275   GATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGC  334
             |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  1167  GATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTATAGTGC  1108

Query  335   CCAACTGAATGCTGGCAACTAAAATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA  394
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1107  CCAACTGAATGCTGGCAACTAAAATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA  1048

Query  395   TCTCACGACACGAGCTGACAACAACCATGCACCACCTGTCACCATTGTCCCCGAAGGGAA  454
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Sbjct  1047  TCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACCACTGTCCCCGAAGGGAA  988

Query  455   AAGTGTATCTCTACCCCGGGCAATGGGATGTCAAAACCTGGTAAGGTTCTTCCCGTTGTT  514
             ||| |||||||||| ||||||| |||||||||| |||||||||||||||| ||||| |
Sbjct  987   AAGCGTATCTCTACACCGGGCAGTGGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCT  928

Query  515   TCAAATTAAACCACTTGcccccccGTTTG-GCGGGcccccccGCCATTCCTTT-AGATTC  572
             || |||||||||| ||| || ||| ||| ||||| |||||  | |||||||| || |||
Sbjct  927   TCCAATTAAACCACATGCTCCACCGCTTGTGCGGG-CCCCCGTCAATTCCTTTGAGTTTC  869

Query  573   ACCTTTGC-GCCGTACTCCCCACGCGGAGTGCTTAATG-GTTAGCTG  617
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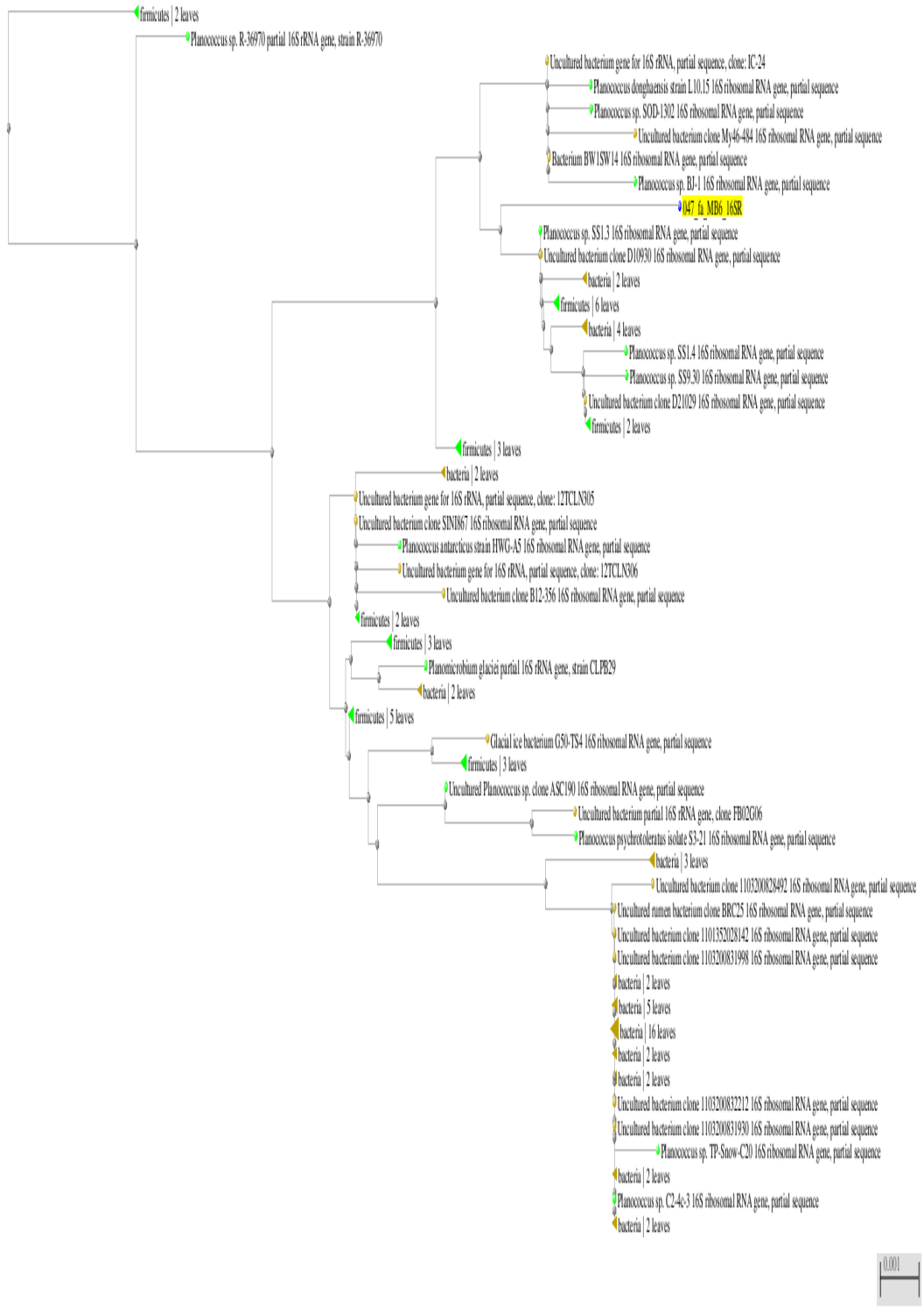
firmicutes | 2 leaves
Planococcus sp. R-36970 partial 16S rRNA gene, strain R-36970
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: IC-24
Planococcus donghaensis strain L10.15 16S ribosomal RNA gene, partial sequence
Planococcus sp. SOD-1302 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone My46-484 16S ribosomal RNA gene, partial sequence
Bacterium BW1SW14 16S ribosomal RNA gene, partial sequence
Planococcus sp. BJ-1 16S ribosomal RNA gene, partial sequence
047_fa_MB6_16SR
Planococcus sp. SS1.3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D10930 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
firmicutes | 6 leaves
bacteria | 4 leaves
Planococcus sp. SS1.4 16S ribosomal RNA gene, partial sequence
Planococcus sp. SS9.30 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D21029 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
firmicutes | 3 leaves
bacteria | 2 leaves
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 12TCLN305
Uncultured bacterium clone SINI867 16S ribosomal RNA gene, partial sequence
Planococcus antarcticus strain HWG-A5 16S ribosomal RNA gene, partial sequence
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 12TCLN306
Uncultured bacterium clone B12-356 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
firmicutes | 3 leaves
Planomicrobium glaciei partial 16S rRNA gene, strain CLPB29
bacteria | 2 leaves
firmicutes | 5 leaves
Glacial ice bacterium G50-TS4 16S ribosomal RNA gene, partial sequence
firmicutes | 3 leaves
Uncultured Planococcus sp. clone ASC190 16S ribosomal RNA gene, partial sequence
Uncultured bacterium partial 16S rRNA gene, clone FB02G06
Planococcus psychrotoleratus isolate S3-21 16S ribosomal RNA gene, partial sequence
bacteria | 3 leaves
Uncultured bacterium clone 1103200828492 16S ribosomal RNA gene, partial sequence
Uncultured rumen bacterium clone BRC25 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1101352028142 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1103200831998 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
bacteria | 5 leaves
bacteria | 16 leaves
bacteria | 2 leaves
bacteria | 2 leaves
Uncultured bacterium clone 1103200832212 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone 1103200831930 16S ribosomal RNA gene, partial sequence
Planococcus sp. TP-Snow-C20 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
Planococcus sp. C2-4c-3 16S ribosomal RNA gene, partial sequence
bacteria | 2 leaves
0.001

Aspergillus sp. OXL4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene,

Sequence ID: gb|KF358714.1| Length: 561 Number of Matches: 1

Range 1: 3 to 538 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
219 bits(242)	3e-53	382/540(71%)	29/540(5%)	Plus/Minus

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Query  1    TCACCTGATAAA-ATTGGTTTGATTTCCGCC-GCCCCCGCCGGCTCTA-AGAGCGTGTGA  57
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Sbjct  538  TCACCTGGAAAAGATTGATTTGCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGA  479

Query  58   CAC--CCCTATACTCTCGAAGATCACACGCTGTGCCCCCTCTGCTTTTGA--CCCCTCCC  113
            ||   ||| |||| ||||| ||||  |||| ||||| || |||| ||||   ||| ||||
Sbjct  478  CAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCC  419

Query  114  CCGCACGGAGAAGGGACCACANCCCC-CANCCNTGCTTTGCTTGATGGGCGACACTGACA  172
            || |  ||||| ||||| ||  |||  ||  |  ||  ||||||||||||  || ||||
Sbjct  418  CCCC--GGAGAGGGGACGACGACCCAACACACAAGCCGTGCTTGATGGGCAGCAATGACG  361

Query  173  CTCGCATGCGCTCGCCACCAGGAATAGGCGGGGTGTGAGTGTGCGTACTCAAACACTC--  230
            |||| |   ||  ||| || ||||||   ||||    | |||||||  ||||| ||||
Sbjct  360  CTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGT--TCAAAGACTCGA  303

Query  231  TGAGTCTTCTACTTCTCACATTCACTATAGTTATTCCCTTTCTTTGCCTTCTTCGCCGGA  290
            ||| ||    | ||||   ||||||  |||||||  | ||||  ||| ||||||  ||
Sbjct  302  TGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGAT  243

Query  291  GCCGGAACATCCATAGCTGTTGTTTATAGTTGGATGTGAGTGAAGCACAAACTACTCTCA  350
            ||||||||    | | |  ||||| | ||||  |  ||| ||    |||| | ||||  |
Sbjct  242  GCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCGATACAATCAACTCAGA  183

Query  351  TTTCACTAAATTATACAGAGTTCCTGGTGACCCCGGACCGCG-------GCGACTGANGT  403
             ||||||| || | ||||||||| ||||| | ||||   |||       | | ||||
Sbjct  182  CTTCACTAGATCAGACAGAGTTCGTGGTGTCTCCGGCGGGCGCGGGCCCGGGGCTGAGAG  123

Query  404  CCCCAGGCCGCCCAGA--GGCAGGCTCGCCGAAGCAAC-AAGGTA-AATAAACACGGGTG  459
            |||| ||| |||  ||  ||| ||| ||||||||||||| |||||| | ||||||||||||
Sbjct  122  CCCCCGGCGGCCATGAATGGCGGGCCCGCCGAAGCAACTAAGGTACAGTAAACACGGGTG  63

Query  460  GGAGGTTGG--ATGGGAGG-GCCCT-CTCTCGGTAATGATCCTTCCGCAGGTTCACCCTA  515
            |||||||||    |  |||  |||| | ||||||||||||||||||||||||||||||||
Sbjct  62   GGAGGTTGGGCTCGCTAGGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCACCCTA  3
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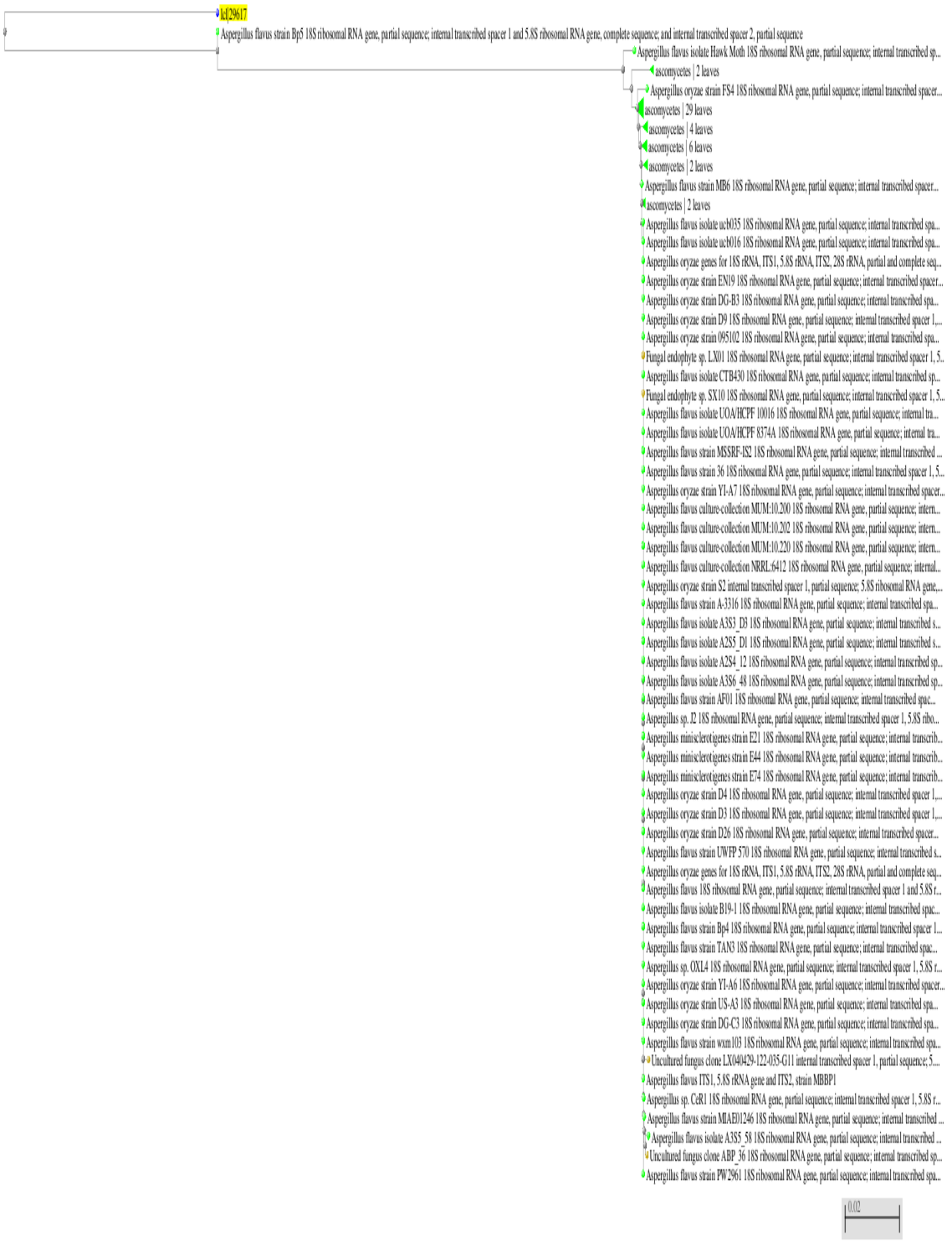
kf129617
Aspergillus flavus strain Bp5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Aspergillus flavus isolate Hawk Moth 18S ribosomal RNA gene, partial sequence; internal transcribed sp...
ascomycetes | 2 leaves
Aspergillus oryzae strain FS4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
ascomycetes | 29 leaves
ascomycetes | 4 leaves
ascomycetes | 6 leaves
ascomycetes | 2 leaves
Aspergillus flavus strain MB6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
ascomycetes | 2 leaves
Aspergillus flavus isolate ucb035 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Aspergillus flavus isolate ucb016 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Aspergillus oryzae genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete seq...
Aspergillus oryzae strain EN19 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
Aspergillus oryzae strain DG-B3 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Aspergillus oryzae strain D9 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...
Aspergillus oryzae strain 095102 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Fungal endophyte sp. LX01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus flavus isolate CTB430 18S ribosomal RNA gene, partial sequence; internal transcribed sp...
Fungal endophyte sp. SX10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus flavus isolate UOA/HCPF 10016 18S ribosomal RNA gene, partial sequence; internal tra...
Aspergillus flavus isolate UOA/HCPF 8374A 18S ribosomal RNA gene, partial sequence; internal tra...
Aspergillus flavus strain MSSRF-I82 18S ribosomal RNA gene, partial sequence; internal transcribed ...
Aspergillus flavus strain 36 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Aspergillus oryzae strain YI-A7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
Aspergillus flavus culture-collection MUM:10.200 18S ribosomal RNA gene, partial sequence; intern...
Aspergillus flavus culture-collection MUM:10.202 18S ribosomal RNA gene, partial sequence; intern...
Aspergillus flavus culture-collection MUM:10.220 18S ribosomal RNA gene, partial sequence; intern...
Aspergillus flavus culture-collection NRRL:6412 18S ribosomal RNA gene, partial sequence; internal...
Aspergillus oryzae strain S2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene,...
Aspergillus flavus strain A-3316 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Aspergillus flavus isolate A3S3_D3 18S ribosomal RNA gene, partial sequence; internal transcribed s...
Aspergillus flavus isolate A2S5_D1 18S ribosomal RNA gene, partial sequence; internal transcribed s...
Aspergillus flavus isolate A2S4_12 18S ribosomal RNA gene, partial sequence; internal transcribed sp...
Aspergillus flavus isolate A3S6_48 18S ribosomal RNA gene, partial sequence; internal transcribed sp...
Aspergillus flavus strain AF01 18S ribosomal RNA gene, partial sequence; internal transcribed spac...
Aspergillus sp. J2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo...
Aspergillus minisclerotigenes strain E21 18S ribosomal RNA gene, partial sequence; internal transcrib...
Aspergillus minisclerotigenes strain E44 18S ribosomal RNA gene, partial sequence; internal transcrib...
Aspergillus minisclerotigenes strain E74 18S ribosomal RNA gene, partial sequence; internal transcrib...
Aspergillus oryzae strain D4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...
Aspergillus oryzae strain D3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...
Aspergillus oryzae strain D26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
Aspergillus flavus strain UWFP 570 18S ribosomal RNA gene, partial sequence; internal transcribed s...
Aspergillus oryzae genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete seq...
Aspergillus flavus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S r...
Aspergillus flavus isolate B19-1 18S ribosomal RNA gene, partial sequence; internal transcribed spac...
Aspergillus flavus strain Bp4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1...
Aspergillus flavus strain TAN3 18S ribosomal RNA gene, partial sequence; internal transcribed spac...
Aspergillus sp. OXL4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S r...
Aspergillus oryzae strain YI-A6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
Aspergillus oryzae strain US-A3 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Aspergillus oryzae strain DG-C3 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Aspergillus flavus strain wxm103 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Uncultured fungus clone LX040429-122-035-G11 internal transcribed spacer 1, partial sequence; 5....
Aspergillus flavus ITS1, 5.8S rRNA gene and ITS2, strain MBBP1
Aspergillus sp. CeR1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S r...
Aspergillus flavus strain MIAE01246 18S ribosomal RNA gene, partial sequence; internal transcribed ...
Aspergillus flavus isolate A3S5_58 18S ribosomal RNA gene, partial sequence; internal transcribed ...
Uncultured fungus clone ABP_36 18S ribosomal RNA gene, partial sequence; internal transcribed sp...
Aspergillus flavus strain PW2961 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
0.02

Penicillium chrysogenum strain MS-02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo

Sequence ID: gb|JX139706.1| Length: 560 Number of Matches: 2

Range 1: 16 to 560 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
948 bits(1050)	0.0	537/545(99%)	0/545(0%)	Plus/Plus

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Query  1    CACCTccccccGTGTTTATTTTACCTTGTTGCTTcggcgggcccgccttaactggccgc  60
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Sbjct  16   CACCTCCCACCCGTGTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGC  75

Query  61   cgggggggcttacgcccccgggcccgcgcccgccgAAGACACCCTCGAACTCTGTCTGAAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  76   CGGGGGGCTTACGCCCCCGGGCCCGCGCCCGCCGAAGACACCCTCGAACTCTGTCTGAAG  135

Query  121  ATTGTAGTCTGAGTGAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTC  180
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Sbjct  136  ATTGTAGTCTGAGTGAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTC  195

Query  181  CGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAA  240
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Sbjct  196  CGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAA  255

Query  241  TCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGA  300
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Sbjct  256  TCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGA  315

Query  301  GCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCCTCCGATCCCGGGGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  316  GCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCCTCCGATCCCGGGGG  375

Query  361  ACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  376  ACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCA  435

Query  421  CCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  436  CCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACC  495

Query  481  TCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAAAGGGATGATTACGCA  540
            |||||||||||||||||||||||||||||||||||||||||||||   ||| || |||  |
Sbjct  496  TCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGGCGGAGGAATACCGA  555

Query  541  GTGAG  545
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Sbjct  556  GTGAG  560
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kf|14209
Penicillium sp. f4 RA-2013 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium sp. QLF72 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured Penicillium ITS1 (partial), 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), clone neuFun18
Penicillium sp. F78 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum isolate TBG3-14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain SCSGAF0049 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain SS-13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium sp. F23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA, partial sequence, strain: JCM 22826
Penicillium chrysogenum isolate PCHRGR191 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum isolate F4-02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain M-10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain HDJZ-ZWM-38 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Penicillium chrysogenum strain H09-024 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain H09-022 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum isolate EN118 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain M-28 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain 101104 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain GZU-BCEC417-2-3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium griseofulvum 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium sp. 36 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium granulatum isolate 732 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured fungus clone 11-44 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...
Penicillium chrysogenum strain H09-100 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium sp. FC1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured fungus clone 11-223 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured Penicillium clone FUN3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain H09-114 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium sp. TXP58 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain M-23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium sp. LF066 genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, isolate LF066
Uncultured Penicillium clone USN8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain GZU-BCECJXF2-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain ATCC 10106 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
ascomycetes | 13 leaves
Penicillium chrysogenum genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, strain TUEF30
Penicillium crustosum isolate SAB-B3B-T internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain DHMJ31 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum isolate BP5T1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum isolate BP2T3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain T19-01 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium crustosum isolate T22-29 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain L1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
ascomycetes | 2 leaves
Penicillium commune strain JJHO internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...
Penicillium chrysogenum strain SL-23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...
Penicillium commune strain N3ZL-43 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and ...
Penicillium commune strain N5ZY-17 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and ...
Penicillium sp. LX 107X-1 genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ge...
Penicillium citrinum strain PTC07 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...
Penicillium sp. SFRh11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal R...
Penicillium chrysogenum strain S58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA ge...
Penicillium sp. M62 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed..
Penicillium chrysogenum strain EN245 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5....
Penicillium commune strain G20-23 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...
Penicillium chrysogenum strain BPLES5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Uncultured fungus clone 11-398 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ri...
ascomycetes | 2 leaves
Penicillium sp. HLS2-6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Hypocrea lixii strain Th 47 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain G6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum isolate NRRL 35635 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain FRR 807 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum ex-type CBS 306.48 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured fungus clone 11-53 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...
ascomycetes | 18 leaves
0.0002

Penicillium crustosum isolate SAB-B3B-T internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal trans

Sequence ID: gb|JX869585.1| Length: 540 Number of Matches: 1

Range 1: 9 to 540 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
949 bits(1052)	0.0	531/533(99%)	1/533(0%)	Plus/Plus

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Query  1    ACCTCCCACCCGTGTTTATTTTACCTTGTTGCTTcggcgggcccgccttaactggccgcc  60
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Sbjct  9    ACCTCCCACCCGTGTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCC  68

Query  61   gggggggcttacgccccgggcccgcgcccgccgAAGACACCCTCGAACTCTGTCTGAAGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  69   GGGGGGCTTACGCCCCGGGCCCGCGCCCGCCGAAGACACCCTCGAACTCTGTCTGAAGA  128

Query  121  TTGTAGTCTGAGTGAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  129  TTGTAGTCTGAGTGAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCC  188

Query  181  GGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  189  GGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAAT  248

Query  241  CATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  249  CATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAG  308

Query  301  CGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCCTCCGATCCCGGGGGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  309  CGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCCTCCGATCCCGGGGGA  368

Query  361  CGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  369  CGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCAC  428

Query  421  CCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  429  CCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCT  488

Query  481  CGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAAGCGGAAGAA  533
            |||||||||||||||||||||||||||||||||||||| ||||||| |||
Sbjct  489  CGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAT-AAGCGGAGGAA  540
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Penicillium chrysogenum strain DHMJ34 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Penicillium chrysogenum strain DHMJ31 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ri...
Hypocrea lixii strain Th 47 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S r...
Paecilomyces penicillatus strain CBS 448.69 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...
Penicillium sp. 274 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S r...
kJ[487703
Penicillium griseofulvum 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inte...
ascomycetes | 2 leaves
ascomycetes | 21 leaves
Uncultured fungus clone 11-398 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal ...
Uncultured Penicillium clone FUN58 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal t...
Uncultured Penicillium clone CEobese390 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ri...
Uncultured fungus clone ABP_42 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal ...
Penicillium chrysogenum strain GZU-BCECA6-2-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
Uncultured fungus clone LJ42885-122-065-B01 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, ...
ascomycetes | 7 leaves
Penicillium chrysogenum DNA for 5.8S ribosomal RNA region
Penicillium chrysogenum ex-type CBS 306.48 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed s...
Penicillium chrysogenum strain CBS 306.48 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; 5.8S ribosomal RNA gene, compl...
Penicillium chrysogenum isolate PCHRGR191 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequen...
Penicillium sp. LF066 genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, isolate LF066
Penicillium sp. 36 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence;...
Penicillium sp. F23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequen...
Penicillium chrysogenum genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA, partial sequence, strain: ICM 22826
Penicillium sp. HLS2-6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequ...
Uncultured Penicillium clone USN8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, c...
Penicillium chrysogenum strain 101104 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer...
Penicillium chrysogenum strain M-23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...
Penicillium chrysogenum strain M-30 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...
Penicillium chrysogenum strain H09-100 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer...
Penicillium chrysogenum genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, strain TUEF27
Penicillium crustosum isolate SAB-B3B-T internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; a...
Penicillium chrysogenum strain SS-13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...
Penicillium chrysogenum strain SCSGAF0049 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed s...
Penicillium chrysogenum isolate TBG3-14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; a...
Penicillium chrysogenum isolate BPZT3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and ...
Penicillium chrysogenum strain GZU-BCECJXF2-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcr...
Uncultured fungus clone 11-223 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, comp...
ascomycetes | 11 leaves
Uncultured Penicillium clone FUN3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S r...
Penicillium chrysogenum strain H09-114 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer...
Penicillium chrysogenum strain H09-022 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer...
Penicillium chrysogenum strain H09-016 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer...
Penicillium chrysogenum strain M-10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...
Penicillium chrysogenum strain M-28 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...
Eurotiomycetes sp. genotype 174 isolate NC0448 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete seque...
Penicillium chrysogenum isolate F4-02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...
ascomycetes | 2 leaves
ascomycetes | 2 leaves
Penicillium chrysogenum strain FRR 807 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer...
Uncultured fungus clone 12-236 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal...
Penicillium chrysogenum isolate NRRL 35635 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribos...
Uncultured fungus clone 11-246 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal...
Penicillium chrysogenum strain ATCC 10106 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed s...
Uncultured fungus clone 11-44 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal ...
Penicillium chrysogenum strain GZU-BCEC417-2-3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcr...
Uncultured fungus clone 11-53 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal ...
ascomycetes | 4 leaves
0.0007

Dendryphiella salina strain CBS 142.60 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5

Sequence ID: gb|DQ411540.1| Length: 583 Number of Matches: 1

Range 1: 1 to 523 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
935 bits(1036)	0.0	522/523(99%)	1/523(0%)	Plus/Minus

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Query  1    ATCCGAGGTCAAA-GTGAGAAAAATGTGGTCTTGATGGATGCTCAACCATGGCTGATCAG  59
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Sbjct  523  ATCCGAGGTCAAAAGTGAGAAAAATGTGGTCTTGATGGATGCTCAACCATGGCTGATCAG  464

Query  60   AAGTGCAAGATTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATCATTTTAAGGCG  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  463  AAGTGCAAGATTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATCATTTTAAGGCG  404

Query  120  AGTCTCGTGAGAGACAAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCT  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403  AGTCTCGTGAGAGACAAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCT  344

Query  180  CGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGAT  239
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Sbjct  343  CGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGAT  284

Query  240  TCACTGAATTCTGCAATTCACACTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCCA  299
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Sbjct  283  TCACTGAATTCTGCAATTCACACTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCCA  224

Query  300  GAACCAAGAGATCCGTTGTTGAAAGTTGTAATAATTACATTGTTTACTGACGCTGATTGC  359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  223  GAACCAAGAGATCCGTTGTTGAAAGTTGTAATAATTACATTGTTTACTGACGCTGATTGC  164

Query  360  AATTACaaaaaaaGGTTTATGGTTGGGTCCTGGTGGCGGGCGAACCCGCCCAGGAAACAA  419
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Sbjct  163  AATTACAAAAAAAGGTTTATGGTTGGGTCCTGGTGGCGGGCGAACCCGCCCAGGAAACAA  104

Query  420  GAAGTGCGCAAAAGACATGGGTGAATAATTCAGACAAGCTGGAGCCCCCACCGAGATGAG  479
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  103  GAAGTGCGCAAAAGACATGGGTGAATAATTCAGACAAGCTGGAGCCCCCACCGAGATGAG  44

Query  480  GTCCCAACCCGCTTTCATATTGTGTAATGATCCCTCCGCAGGT  522
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Sbjct  43   GTCCCAACCCGCTTTCATATTGTGTAATGATCCCTCCGCAGGT  1
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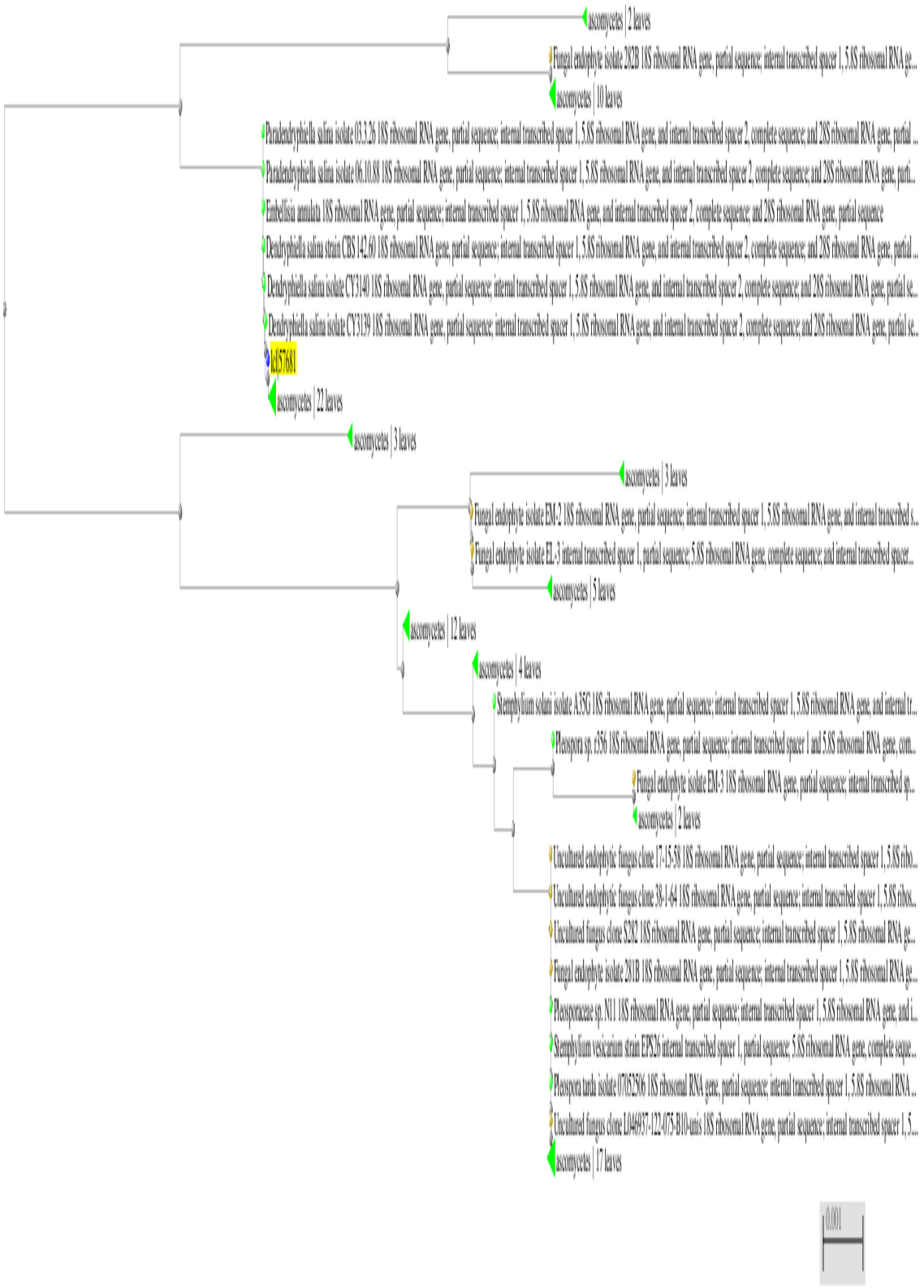
ascomycetes | 2 leaves
Fungal endophyte isolate 2828 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge...
ascomycetes | 10 leaves
Paradendryphiella salina isolate 03.3.26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial ...
Paradendryphiella salina isolate 06.10.88 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, parti...
Embellisia annulata 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Dendryphiella salina strain CBS 142.60 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial ...
Dendryphiella salina isolate CY3140 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial se...
Dendryphiella salina isolate CY3139 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial se...
KM577681
ascomycetes | 22 leaves
ascomycetes | 3 leaves
ascomycetes | 3 leaves
Fungal endophyte isolate EM-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed s...
Fungal endophyte isolate EL-3 internal transcribed spacer 1, partial sequence;5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer...
ascomycetes | 5 leaves
ascomycetes | 12 leaves
ascomycetes | 4 leaves
Stemphylium solani isolate A35G 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr...
Plenospora sp. r356 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, com...
Fungal endophyte isolate EM-3 18S ribosomal RNA gene, partial sequence; internal transcribed sp...
ascomycetes | 2 leaves
Uncultured endophytic fungus clone 17-15-58 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo...
Uncultured endophytic fungus clone 30-1-64 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribos...
Uncultured fungus clone S202 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge...
Fungal endophyte isolate 281B 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge...
Plenosporaceae sp. N11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and i...
Stemphylium vesicarium strain EPS26 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete seque...
Plenospora tarda isolate 0705252b6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ...
Uncultured fungus clone EM4937-122-07S-B10-unis 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5....
ascomycetes | 17 leaves
0.001

Alternaria tenuissima strain AL-33 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S

Sequence ID: gb|KC341977.1| Length: 578 Number of Matches: 1

Range 1: 24 to 577 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
987 bits(1094)	0.0	552/554(99%)	1/554(0%)	Plus/Plus

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Query  1    GTGGTTTGCTGGCA-CAGCGTCCGCCCCAAGTATTTTTCACCCATGTCTTTTGCGCACTT  59
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Sbjct  24   GTGGTTTGCTGGCAACAGCGTCCGCCCCAAGTATTTTTCACCCATGTCTTTTGCGCACTT  83

Query  60   TTTGTTTCCTGGGCGAGTTCGCTCGCCACCAGGACCCAACCATAAACCttttttATGCA  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  84   TTTGTTTCCTGGGCGAGTTCGCTCGCCACCAGGACCCAACCATAAACCTTTTTTTATGCA  143

Query  120  GTTGCAATCAGCGTCAGTATAATAATTCAATTTATTAAAACTTTCAACAACGGATCTCTT  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  144  GTTGCAATCAGCGTCAGTATAATAATTCAATTTATTAAAACTTTCAACAACGGATCTCTT  203

Query  180  GGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTC  239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204  GGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTC  263

Query  240  AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCT  299
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  264  AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCT  323

Query  300  GTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTTTTGTCTCTCC  359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  324  GTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTTTTGTCTCTCC  383

Query  360  CCTTGTTGGGGGAGACTCGCCTTAAAACGATTGGCAGCCGACCTACTGGTTTTCGGAGCG  419
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  384  CCTTGTTGGGGGAGACTCGCCTTAAAACGATTGGCAGCCGACCTACTGGTTTTCGGAGCG  443

Query  420  CAGCACAAATTTGCGCCTTCCAATCCACGGGGCGGCATCCAGCAAGCCTTTGTTTTCTAT  479
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  444  CAGCACAAATTTGCGCCTTCCAATCCACGGGGCGGCATCCAGCAAGCCTTTGTTTTCTAT  503

Query  480  AACAAATCCACATTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCA  539
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  504  AACAAATCCACATTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCA  563

Query  540  ATAAGCCGGAGGAA  553
            ||||| ||||||||
Sbjct  564  ATAAGGCGGAGGAA  577
```


Alternaria sp. TA26-52 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; a...
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, strain U...
ascomycetes | 6 leaves
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 2...
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 2...
Exserohilum rostratum internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra...
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, ...
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, ...
Uncultured fungus clone f2HSc17 18S ribosomal RNA gene, partial sequence; internal transcri...
ascomycetes | 2 leaves
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 2...
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 2...
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 2...
Fungal sp. F234 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, comp...
ascomycetes | 3 leaves
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S...
Uncultured fungus clone CMH085 18S ribosomal RNA gene, partial sequence; internal transcribed...
Exserohilum sp. P26E1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
Exserohilum sp. CPO 10.011 18S ribosomal RNA gene, partial sequence; internal transcribed spa...
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S...
Setosphaeria rostrata genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S...
Fungal endophyte sp. ZY-2009 isolate path2 18S ribosomal RNA gene, partial sequence; internal t...
Setosphaeria rostrata isolate ExRo 2 18S ribosomal RNA gene, partial sequence; internal transcrib...
ascomycetes | 15 leaves
ascomycetes | 50 leaves
Setosphaeria rostrata strain EGJMP10 18S ribosomal RNA gene, partial sequence
Setosphaeria rostrata strain P3006008 18S ribosoamal RNA gene, partial sequence; internal transcr...
Exserohilum rostratum 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...
gi|24155
Setosphaeria rostrata strain Ex 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...
0.002

Bacillus pumilus strain XJSL5-8 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|GQ903427.1| Length: 1512 Number of Matches: 1

Range 1: 34 to 697 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1103 bits(1222)	0.0	645/665(97%)	2/665(0%)	Plus/Plus

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Query  2    GGCGTGCCTAATACTTGCAAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTACCG  61
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Sbjct  34   GGCGTGCCTAATACATGCAAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCG  93

Query  62   GCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAAC  121
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  94   GCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAAC  153

Query  122  CGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGG  181
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  154  CGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGG  213

Query  182  CTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAA  241
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  214  CTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAA  273

Query  242  GGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCC  301
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  274  GGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCC  333

Query  302  CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAG  361
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  334  CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAG  393

Query  362  CAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACA  421
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  394  CAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACA  453

Query  422  AGTGCAAGAGTAACTGCTTGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTAC  481
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  482  GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAA  541
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Query  661  AAATG  665
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Sbjct  693  AAATG  697
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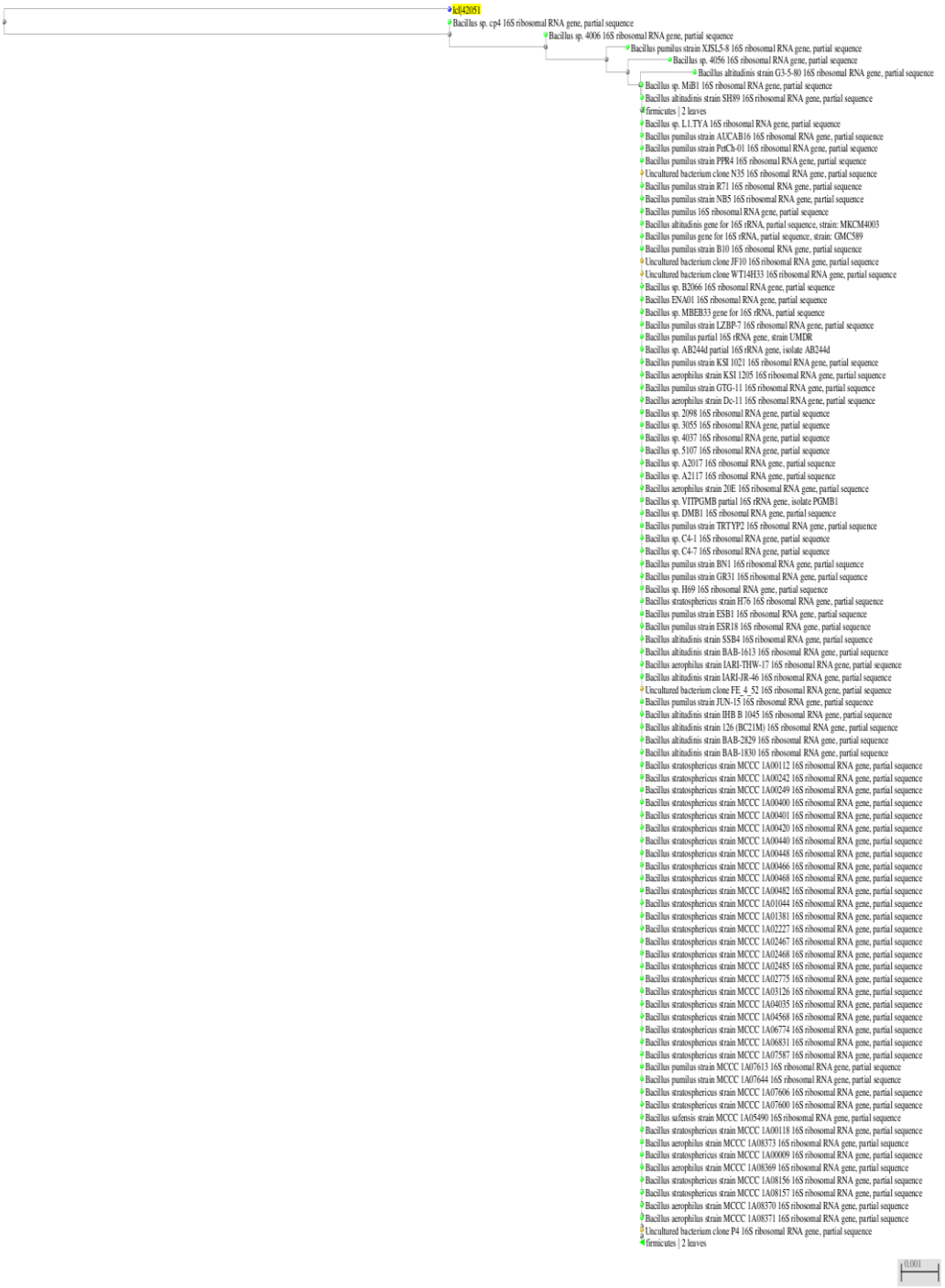
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Bacillus altitudinis strain G3-5-80 16S ribosomal RNA gene, partial sequence
Bacillus sp. MiB1 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH89 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
Bacillus sp. L1.TYA 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain AUCAB16 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain PetCh-01 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain PPR4 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone N35 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain R71 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain NB5 16S ribosomal RNA gene, partial sequence
Bacillus pumilus 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis gene for 16S rRNA, partial sequence, strain: MKCM4003
Bacillus pumilus gene for 16S rRNA, partial sequence, strain: GMC589
Bacillus pumilus strain B10 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone JF10 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone WT14H33 16S ribosomal RNA gene, partial sequence
Bacillus sp. B2066 16S ribosomal RNA gene, partial sequence
Bacillus ENA01 16S ribosomal RNA gene, partial sequence
Bacillus sp. MBEB33 gene for 16S rRNA, partial sequence
Bacillus pumilus strain LZBP-7 16S ribosomal RNA gene, partial sequence
Bacillus pumilus partial 16S rRNA gene, strain UMDR
Bacillus sp. AB244d partial 16S rRNA gene, isolate AB244d
Bacillus pumilus strain KSI 1021 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain KSI 1205 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GTG-11 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain Dc-11 16S ribosomal RNA gene, partial sequence
Bacillus sp. 2098 16S ribosomal RNA gene, partial sequence
Bacillus sp. 3055 16S ribosomal RNA gene, partial sequence
Bacillus sp. 4037 16S ribosomal RNA gene, partial sequence
Bacillus sp. 5107 16S ribosomal RNA gene, partial sequence
Bacillus sp. A2017 16S ribosomal RNA gene, partial sequence
Bacillus sp. A2117 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain 20E 16S ribosomal RNA gene, partial sequence
Bacillus sp. VITPGMB partial 16S rRNA gene, isolate PGMB1
Bacillus sp. DMB1 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain TRTYP2 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-1 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-7 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain BN1 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR31 16S ribosomal RNA gene, partial sequence
Bacillus sp. H69 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain H76 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESB1 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESR18 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SSB4 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1613 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain IARI-THW-17 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IARI-JR-46 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone FE_4_52 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain JUN-15 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IHB B 1045 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain 126 (BC21M) 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-2829 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1830 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00112 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00242 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00249 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00400 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00401 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00420 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00440 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00448 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00466 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00468 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00482 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A01044 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A01381 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02227 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02467 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02468 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02485 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02775 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A03126 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A04035 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A04568 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A06774 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A06831 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A07587 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain MCCC 1A07613 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain MCCC 1A07644 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A07606 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A07600 16S ribosomal RNA gene, partial sequence
Bacillus safensis strain MCCC 1A05490 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00118 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain MCCC 1A08373 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00009 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain MCCC 1A08369 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A08156 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A08157 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain MCCC 1A08370 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain MCCC 1A08371 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone P4 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
0.001

Bacillus aerophilus strain MCCC 1A08370 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|JX680140.1| Length: 1513 Number of Matches: 1

Range 1: 36 to 776 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1274 bits(1412)	0.0	727/741(98%)	0/741(0%)	Plus/Plus

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Query  61   GGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCG  120
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Sbjct  96   GGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCG  155

Query  121  GAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCT  180
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Sbjct  336  GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA  395

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Sbjct  756  TGAGGAGCGAAAGCGTGGGGA  776
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kf9391
Bacillus pumilus strain GPL15 16S ribosomal RNA gene, partial sequence
Bacillus sp. 4056 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02775 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02485 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02468 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02467 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A02227 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A01381 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A01044 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00482 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00468 16S ribosomal RNA gene, partial sequence
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Bacillus stratosphericus strain MCCC 1A00448 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00440 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00420 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00401 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00400 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00249 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00242 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00112 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1830 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-2829 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain 126 (BC21M) 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IHB B 1045 16S ribosomal RNA gene, partial sequence
Bacillus sp. PS-62 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain JUN-15 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone FE_4_52 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IARI-JR-46 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain IARI-THW-17 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1613 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SSB4 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESR18 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESB1 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain H76 16S ribosomal RNA gene, partial sequence
Bacillus sp. H69 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR31 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain BN1 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-7 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-1 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain TRTYP2 16S ribosomal RNA gene, partial sequence
Bacillus sp. VITPGMB partial 16S rRNA gene, isolate PGMB1
Bacillus aerophilus strain 20E 16S ribosomal RNA gene, partial sequence
Bacillus sp. A2117 16S ribosomal RNA gene, partial sequence
Bacillus sp. A2017 16S ribosomal RNA gene, partial sequence
Bacillus sp. 5107 16S ribosomal RNA gene, partial sequence
Bacillus sp. 4037 16S ribosomal RNA gene, partial sequence
Bacillus sp. 2098 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH89 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GTG-11 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain KSI 1205 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain KSI 1021 16S ribosomal RNA gene, partial sequence
Bacillus sp. AB244d partial 16S rRNA gene, isolate AB244d
Bacillus pumilus partial 16S rRNA gene, strain UMDR
Bacillus pumilus strain LZBP-7 16S ribosomal RNA gene, partial sequence
Bacillus sp. MBEB33 gene for 16S rRNA, partial sequence
Bacillus ENA01 16S ribosomal RNA gene, partial sequence
Bacillus sp. B2066 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain B10 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis gene for 16S rRNA, partial sequence, strain: MKCM4003
Bacillus pumilus 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain NB5 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain R71 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone N35 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain KB66 16S ribosomal RNA gene, partial sequence
Bacillus pumilus gene for 16S rRNA, partial sequence, strain: NBRC 12087
Bacillus pumilus strain CJ-S-R2A7 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain PPR4 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain PetCh-01 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain AUCAB16 16S ribosomal RNA gene, partial sequence
Bacillus sp. L1.TYA 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain AIMST KBT13-X 16S ribosomal RNA gene, partial sequence
Bacillus sp. S2ID26(2011) 16S ribosomal RNA gene, partial sequence
Bacillus sp. MiB1 16S ribosomal RNA gene, partial sequence
Bacillus sp. HL-5 16S ribosomal RNA gene, partial sequence
Uncultured bacterium partial 16S rRNA gene, clone JX96
firmicutes | 3 leaves
Bacillus stratosphericus strain MCCC 1A03126 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A04035 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A04568 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A06774 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A06831 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A07587 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain MCCC 1A07613 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain MCCC 1A07644 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A07606 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A07600 16S ribosomal RNA gene, partial sequence
Bacillus safensis strain MCCC 1A05490 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00118 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain MCCC 1A08373 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00009 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain MCCC 1A08369 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A08156 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A08157 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain MCCC 1A08370 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain MCCC 1A08371 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone P4 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
0.001

Bacillus altitudinis strain SH148 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KC172054.1| Length: 1453 Number of Matches: 1

Range 1: 729 to 1450 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1209 bits(1340)	0.0	705/725(97%)	3/725(0%)	Plus/Minus

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Query  2     GGCCCCCCTTTCGTGCAGCAGGGCTCCATAAAGGTTACCTCACCGACTTCGGGTGTTGCA  61
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Sbjct  1450  GGCCCACCTTTCG-GCGGCTGG-CTCCATAAAGGTTACCTCACCGACTTCGGGTGTTGCA  1393

Query  62    AACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATG  121
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Sbjct  1392  AACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATG  1333

Query  122   CTGATCCGCGATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGA  181
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Sbjct  1332  CTGATCCGCGATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGA  1273

Query  182   ACTGAGAACAGATTTGTGGGATTGGCTAAACCTTGCGGTCTCGCAGCCCTTTGTTCTGTC  241
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Sbjct  1272  ACTGAGAACAGATTTGTGGGATTGGCTAAACCTTGCGGTCTCGCAGCCCTTTGTTCTGTC  1213

Query  242   CATTGTANCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCAC  301
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Sbjct  1212  CATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCAC  1153

Query  302   CTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAA  361
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Sbjct  1152  CTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAA  1093

Query  362   GATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGAC  421
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Query  422   AACCATGCACCACCTGTCACTCTGTCCCCGAAGGGAAAGCCCTATCTCTAGGGTTGTCAG  481
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Sbjct  1032  AACCATGCACCACCTGTCACTCTGTCCCCGAAGGGAAAGCCCTATCTCTAGGGTTGTCAG  973

Query  482   AGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACC  541
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Query  542   GCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCACTCTTGCGACCGTACTCCCCAGGC  601
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Sbjct  912   GCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGC  853

Query  602   GGAGTGCTTAATGCGTTAGNTGCAGCACTAAAAGGGCGGAAACCCCCTAACACTTATCAC  661
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Sbjct  852   GGAGTGCTTAATGCGTTAGCTGCAGCACT-AAGGGGCGGAAACCCCCTAACACTTAGCAC  794

Query  662   TCATCGTTTACAGCGTGGAATACCACGGGATCAAATCCTGTTTGCTCCCCACACTTTTTC  721
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Sbjct  793   TCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGC  734

Query  722   TCCTC  726
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Sbjct  733   TCCTC  729
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dq59795
Bacillus sp. KtMA2-7 16S ribosomal RNA gene, partial sequence
Bacillus sp. PB3(2006) 16S ribosomal RNA gene, partial sequence
firmicutes | 4 leaves
Bacillus altitudinis strain SH124 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain DF 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH89 16S ribosomal RNA gene, partial sequence
Bacillus sp. JSM 111006 16S ribosomal RNA gene, partial sequence
Bacillus sp. MiB1 16S ribosomal RNA gene, partial sequence
Bacillus sp. 34386CBRRJ 16S ribosomal RNA gene, partial sequence
Bacillus sp. GC-4 16S ribosomal RNA gene, partial sequence
Bacillus sp. TSWCS4 16S ribosomal RNA gene, partial sequence
Bacillus sp. 28KZ 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain :41KF2a 16S ribosomal RNA, partial sequence
Bacillus aerophilus strain :28K 16S ribosomal RNA, partial sequence
Bacillus altitudinis strain SH109 16S ribosomal RNA gene, partial sequence
Bacillus sp. SG9 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain SH88 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH148 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain SH83 16S ribosomal RNA gene, partial sequence
Bacterium 8-gw3-8 16S ribosomal RNA gene, partial sequence
firmicutes | 10 leaves
Bacillus stratosphericus strain L47 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
firmicutes | 5 leaves
firmicutes | 2 leaves
Bacillus pumilus strain LS30 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis gene for 16S rRNA, partial sequence, strain: MKCM4003
Bacillus sp. 13835 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00008 16S ribosomal RNA gene, partial sequence
Bacillus sp. Gr-11 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1830 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain BAB-214 16S ribosomal RNA gene, partial sequence
Bacillus sp. C63 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IHB B 1045 16S ribosomal RNA gene, partial sequence
Bacillus sp. AHBR17 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GC51 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GC43 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1613 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESR21 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SSB4 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML484 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML211 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML252 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML256 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML353 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESR18 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESR14 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESB1 16S ribosomal RNA gene, partial sequence
Bacillus sp. S48 16S ribosomal RNA gene, partial sequence
Bacillus sp. H69 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain B3 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain B12 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-7 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone SV-10 16S ribosomal RNA gene, partial sequence
firmicutes | 26 leaves
Uncultured bacterium clone P4 16S ribosomal RNA gene, partial sequence
0.001

Bacillus sp. Gr-11 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KF010630.1| Length: 1485 Number of Matches: 1

Range 1: 766 to 1427 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1186 bits(1314)	0.0	660/662(99%)	0/662(0%)	Plus/Minus

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Query  19    GCTCGATAGAGGTTACCTCACCGACTTCGGGTGTTGCAAACTCTCGTGGTGTGACGGGCG  78
             |||| ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1427  GCTCCATAAAGGTTACCTCACCGACTTCGGGTGTTGCAAACTCTCGTGGTGTGACGGGCG  1368

Query  79    GTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGAT  138
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1367  GTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGAT  1308

Query  139   TCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGAT  198
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1307  TCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGAT  1248

Query  199   TGGCTAAACCTTGCGGTCTCGCAGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCCC  258
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1247  TGGCTAAACCTTGCGGTCTCGCAGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCCC  1188

Query  259   AGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGC  318
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1187  AGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGC  1128

Query  319   AGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGC  378
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1127  AGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGC  1068

Query  379   GGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTC  438
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1067  GGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTC  1008

Query  439   TGTCCCCGAAGGGAAAGCCCTATCTCTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGG  498
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1007  TGTCCCCGAAGGGAAAGCCCTATCTCTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGG  948

Query  499   TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAAT  558
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  947   TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAAT  888

Query  559   TCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTG  618
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887   TCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTG  828

Query  619   CAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTAC  678
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  827   CAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTAC  768

Query  679   CA  680
             ||
Sbjct  767   CA  766
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kcl51887
firmicutes | 12 leaves
Bacillus aerophilus strain SH31 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain SH61 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain SH83 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain SH88 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH89 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH91 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain SH97 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH109 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH124 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain SH130 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain SH143 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH148 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH156 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH161 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SH172 16S ribosomal RNA gene, partial sequence
Bacillus sp. B26(2012) 16S ribosomal RNA gene, partial sequence
Bacillus sp. JC3 16S ribosomal RNA gene, partial sequence
Bacillus sp. DF4(2012) 16S ribosomal RNA gene, partial sequence
Bacterium BW3SW13 16S ribosomal RNA gene, partial sequence
Paenibacillus polymyxa strain SF-19 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain X-11 16S ribosomal RNA gene, partial sequence
Bacillus sp. 5101 16S ribosomal RNA gene, partial sequence
Bacillus sp. 5120 16S ribosomal RNA gene, partial sequence
Bacillus pumilus 16S ribosomal RNA gene, partial sequence
Bacillus sp. XJ9DR1 gene for 16S rRNA, partial sequence
Bacillus sp. XJ9DR2 gene for 16S rRNA, partial sequence
Bacillus sp. XJ9DNR2 gene for 16S rRNA, partial sequence
Bacillus sp. VITPGMB partial 16S rRNA gene, isolate PGMB1
Bacillus pumilus strain M7 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain OC4 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone CDX31 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain MD 02 16S ribosomal RNA gene, partial sequence
Bacillus sp. DMB1 16S ribosomal RNA gene, partial sequence
Bacillus sp. JS3 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain Z4S2 16S ribosomal RNA gene, partial sequence
Bacillus aerophilus strain B65 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone SV-10 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-3 16S ribosomal RNA gene, partial sequence
Bacillus sp. C4-7 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain B12 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C72 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C158 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain V1 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain B3 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain Nsic-2 16S ribosomal RNA gene, partial sequence
Bacillus subtilis strain T75 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain T86 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain T246 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR24 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR25 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR27 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR28 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GR36 16S ribosomal RNA gene, partial sequence
Bacillus sp. Dca21 16S ribosomal RNA gene, partial sequence
Bacillus sp. Dca24 16S ribosomal RNA gene, partial sequence
Bacillus sp. H69 16S ribosomal RNA gene, partial sequence
Bacillus sp. S48 16S ribosomal RNA gene, partial sequence
Bacillus sp. S90 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML256 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML252 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML211 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ML484 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain SSB4 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain DF 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain EkA2-2 16S ribosomal RNA gene, partial sequence
Bacillus sp. KtRA5-106 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain ESR21 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain KtRA5-79 16S ribosomal RNA gene, partial sequence
Bacillus sp. KtTA4-17 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain KtMA2-6 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1613 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain IARI-THW-8 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IARI-JR-46 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GC43 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain GC51 16S ribosomal RNA gene, partial sequence
Bacillus pumilus strain MS42 16S ribosomal RNA gene, partial sequence
Bacillus sp. EkMoC1-M1 16S ribosomal RNA gene, partial sequence
Bacillus sp. AHBR17 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain IHB B 1045 16S ribosomal RNA gene, partial sequence
Bacterium R-3 16S ribosomal RNA gene, partial sequence
Bacillus sp. C63 16S ribosomal RNA gene, partial sequence
Bacillus sp. CZB12 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain 126 (BC21M) 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain BAB-214 16S ribosomal RNA gene, partial sequence
Bacillus altitudinis strain BAB-1830 16S ribosomal RNA gene, partial sequence
Bacillus sp. Gr-11 16S ribosomal RNA gene, partial sequence
Bacillus stratosphericus strain MCCC 1A00008 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone P4 16S ribosomal RNA gene, partial sequence
0.0001

- **phylogenetic analysis of fungi and bacteria related to chitin**

Trichoderma citrinoviride strain T13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RN

Sequence ID: gb|HQ596929.1| Length: 621 Number of Matches: 1

Range 1: 49 to 621 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1007 bits(1116)	0.0	569/574(99%)	2/574(0%)	Plus/Plus

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Query  1    AACCCAATGTGA-CGTTACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCG  59
            ||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  49   AACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCG  108

Query  60   CAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCTAAAACTCTTATTGTATACCCCCT  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  109  CAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCTAAAACTCTTATTGTATACCCCCT  168

Query  120  CGCGGGttttttATAATCTGAGCCTTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGA  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  169  CGCGGGTTTTTTTATAATCTGAGCCTTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGA  228

Query  180  ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATG  239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  229  ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATG  288

Query  240  CGATAAGTAATGTGAATTGCANAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG  299
            |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  289  CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG  348

Query  300  CCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCC  359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  349  CCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCC  408

Query  360  GGGGGGTCGGCGTTGGGGATCGGCCCTCCCTTAGCGGGTGGCCGTCTCCGAAATACAGTG  419
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  409  GGGGGGTCGGCGTTGGGGATCGGCCCTCCCTTAGCGGGTGGCCGTCTCCGAAATACAGTG  468

Query  420  GCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACACTCGCATCGGGAGCGCGGCG  479
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  469  GCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACACTCGCATCGGGAGCGCGGCG  528

Query  480  CGTCCACAGCCGTTAAACACCCAACTTCTGAAATGTTGACCTCGGATCAGGTAGGAATAC  539
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529  CGTCCACAGCCGTTAAACACCCAACTTCTGAAATGTTGACCTCGGATCAGGTAGGAATAC  588

Query  540  CCGCTGAACTTAAGCATATCAAAAGGCGGGAGGA  573
            ||||||||||||||||||||| | || ||||||
Sbjct  589  CCGCTGAACTTAAGCATATCAATAAGC-GGAGGA  621
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Fungal sp. ARIZ B131 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S...
Hypocrea lixii genomic DNA containing ITS1, 5.8S rRNA gene and ITS2, strain T-22
Hypocrea lixii strain JB T1244 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence;...
Hypocrea lixii strain DAOM 229971 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq...
Fungal sp. ARIZ B104 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B418 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B423 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B453 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1039 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
Hypocrea lixii isolate C40065P301CC594 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, p...
Trichoderma harzianum strain T-alces internal transcribed spacer 1, partial sequence; and 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 28S ribosomal RNA gene, comple...
Hypocrea lixii strain TUB F-961 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Hypocrea lixii strain TUB F-444 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Trichoderma harzianum strain TUB F-834 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Hypocreomycetidae sp. SHW16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA ...
Hypocrea lixii isolate SZMC 20854 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal ...
Trichoderma sp. Ir.504 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 2...
Hypocrea lixii isolate SZMC 20853 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal ...
Hypocrea lixii strain GJS 04-71 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequ...
Hypocreomycetidae sp. SHW14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosom...
ascomycetes | 2 leaves
Hypocrea lixii isolate SZMC 20857 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ri...
Trichoderma harzianum strain TR274 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence;...
fungi | 2 leaves
Trichoderma harzianum isolate 8238 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, ...
ascomycetes | 6 leaves
Hypocrea lixii isolate OTU660 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed sp...
Hypocrea lixii isolate NBAII TN-11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...
kcl|63867
Hypocrea lixii isolate R-30 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal...
ascomycetes | 4 leaves
Hypocreomycetidae sp. SHW02 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer...
Fungal sp. ARIZ B511.p 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tran...
Trichoderma harzianum strain T-soybean internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosom...
Fungal sp. ARIZ B007 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B403 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Hypocrea lixii strain DAOM 231402 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq...
Fungal sp. ARIZ B508 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Trichoderma sp. 4 BRO-2013 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; ...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1094 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1215 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1024 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1010 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1161 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
Hypocrea lixii strain TUB F-1020 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Trichoderma sp. HYKH3/C1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; a...
Trichoderma sp. HYKH4/C1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; a...
Hypocrea lixii strain IMI 359823 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Hypocrea lixii isolate hz-68 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Hypocrea lixii strain DAOM 231646 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq...
Hypocrea lixii strain JB T2181 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence;...
Fungal sp. ARIZ B511 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B502 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B474 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B473 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B447 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B446cA 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and ...
Fungal sp. ARIZ B442 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B440 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B414cA 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and ...
Fungal sp. ARIZ B408 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B402cB 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and ...
Fungal sp. ARIZ B402cA 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and ...
Fungal sp. ARIZ B261 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B137 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B097 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B094 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B092 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B089 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B056 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
Fungal sp. ARIZ B031 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2...
ascomycetes | 4 leaves
Hypocrea sp. 3 TMS-2011 voucher MS3p_50-23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...
Hypocrea lixii genomic DNA containing ITS1, 5.8S rRNA gene and ITS2, strain T-78
Hypocrea lixii strain DAOM 222149 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq...
Trichoderma harzianum strain TUB F-1078 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Hypocrea lixii strain DAOM 220786 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq...
Hypocrea lixii strain A21 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and ...
Hypocrea lixii strain A23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and ...
Hypocrea lixii isolate HA1103 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence;...
Trichoderma citrinoviride strain T36 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq...
Trichoderma citrinoviride strain T13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq...
Hypocrea lixii genomic DNA containing ITS1, 5.8S rRNA gene and ITS2, strain T-4
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1049 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1248 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
ascomycetes | 2 leaves
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1224 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans...
0.0009

Fusarium sp. 201k internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed space

Sequence ID: gb|JX244004.1| Length: 1040 Number of Matches: 2

Range 1: 42 to 521 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
691 bits(766)	0.0	443/480(92%)	2/480(0%)	Plus/Plus

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Query  14   ACTTGTTGCCTCCGCGGATGGATCCCCTCCCGGGGGTG-CGGGAAGGCCCGCACCAGGACC  72
            |||||||||||| ||||||    || |||||||     ||||| |||||||   ||||||
Sbjct  42   ACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACC  101

Query  73   CCTACCCTCTGATTCTATATGTATCTTCTGATTAACACCCTCAATAAATCTTAACTTTCA  132
            ||||  ||||| |||||||||||| ||||||| ||| ||| | ||||||||  ||||||||
Sbjct  102  CCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCA  161

Query  133  ACAACGGATCCCTTGGTTCTGGCATCAATGAATAACGCAACAAAATGCGATAAATAATGT  192
            |||||||||| ||||||||||||||| ||||| |||||| ||||||||||||| ||||||
Sbjct  162  ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGT  221

Query  193  GAATTGCATAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTC  252
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Sbjct  222  GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTC  281

Query  253  TGGCGGGCATGCCTGTTCGAGCGTCATTTCA-CCCTCATGCACACCTTGGTGTTGGGACT  311
            |||||||||||||||||||||||||||||| |||||| ||||| ||||||||||||||||
Sbjct  282  TGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACT  341

Query  312  CGCGTTAATTCTCGTTCCTCAAATTGATTGGCGGTCACGTCCCGCTTCCATAGCGTAGTA  371
            |||||||||| |||||||||||||||||||||||||||  ||||||||||||||||||
Sbjct  342  CGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTA  401

Query  372  GTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATG  431
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  402  GTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATG  461

Query  432  TTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAA  491
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  462  TTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAA  521
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kl|57307
Fusarium oxysporum isolate GIFUDHAN8 internal transcribed spacer 1, partial sequence; 5.8S ribosom..
Fusarium oxysporum isolate F49 18S ribosomal RNA gene, partial sequence; internal transcribed ...
Uncultured soil fungus clone 53-17 18S ribosomal RNA gene, partial sequence; internal tr...
ascomycetes | 4 leaves
Fusarium oxysporum f. sp. melonis 18S ribosomal RNA gene, partial sequence; internal trans...
ascomycetes | 3 leaves
Fusarium oxysporum isolate Endophyte 60 18S ribosomal RNA gene, partial sequence; inter...
Fusarium sp. NRRL 43466 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...
Fusarium sp. NRRL 43454 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...
Fusarium sp. M3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...
Fusarium sp. F55 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F13 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...
Fusarium sp. F15 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F48 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F43 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F30 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F31 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F53 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F36 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F38 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F41 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F42 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F23 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F47 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F21 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F35 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...
Fusarium sp. F57 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F71 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F60 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F26 internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed..
Fusarium oxysporum f. sp. benincasae internal transcribed spacer 1, partial sequence; 5.8S ri...
Fusarium sp. WF152 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
Colletotrichum sp. WF154 18S ribosomal RNA gene, partial sequence; internal transcribed s...
Uncultured fungus clone Hyp18 18S ribosomal RNA gene, partial sequence; internal transcri...
Fusarium oxysporum genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, IT...
Fusarium sp. BESC883b internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...
Fusarium oxysporum genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, IT...
Fusarium sp. WF157 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
Fusarium sp. WF153 18S ribosomal RNA gene, partial sequence; internal transcribed spacer...
Fusarium sp. Lu5-1 XZ-2010 18S ribosomal RNA gene, partial sequence; internal transcribed...
Fusarium sp. 14018 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...
Fusarium sp. F9 internal transcribed spacer 1, partial sequence; and 5.8S ribosomal RNA ge...
Fusarium sp. F74 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium sp. F59 internal transcribed space 1 gene, partial sequence; 5.8S ribosomal RNA ge...
Fusarium oxysporum strain HBFO01 internal transcribed spacer 1, partial sequence; 5.8S ri...
Fusarium oxysporum strain JAI-MB24 18S ribosomal RNA gene, partial sequence; internal t...
Fusarium sp. 3-4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...
Fusarium oxysporum genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, IT...
Fusarium oxysporum isolate F1 internal transcribed spacer 1, partial sequence; 5.8S ribosom...
Fusarium oxysporum strain 70 internal transcribed spacer 1, partial sequence; 5.8S ribosomal...
Fusarium oxysporum isolate K5 internal transcribed spacer 1, partial sequence; 5.8S ribosom...
Fusarium oxysporum strain FOCCB-7 18S ribosomal RNA gene, partial sequence; internal tr...
Fusarium oxysporum strain FOCCB-6 18S ribosomal RNA gene, partial sequence; internal tr...
Fusarium oxysporum strain FOCCB-5 18S ribosomal RNA gene, partial sequence; internal tr...
Fusarium oxysporum strain FOCCB-1 18S ribosomal RNA gene, partial sequence; internal tr...
Fusarium oxysporum strain FUS ITS 07 18S ribosomal RNA gene, partial sequence; internal...
Fusarium oxysporum isolate GIFUDHAN6 internal transcribed spacer 1, partial sequence; 5....
Fusarium oxysporum isolate GIFUDHAN5 internal transcribed spacer 1, partial sequence; 5....
Fungal endophyte strain 1044 18S ribosomal RNA gene, partial sequence; internal transcribed..
Fusarium oxysporum isolate 12168 internal transcribed spacer 1, partial sequence; 5.8S ribo...
Fusarium oxysporum isolate FoxySIN1 internal transcribed spacer 1, partial sequence; 5.8S r...
Fusarium oxysporum strain FOCCB-3 internal transcribed spacer 1, partial sequence; 5.8S ri...
Fungal endophyte strain 1146 18S ribosomal RNA gene, partial sequence; internal transcribed..
Fusarium oxysporum NBAIM:1281 internal transcribed spacer 1, partial sequence; 5.8S ribo...
Fusarium oxysporum isolate Fol-km internal transcribed spacer 1, partial sequence; 5.8S ribo...
Uncultured endophytic fungus clone R3-63 18S ribosomal RNA gene, partial ...
Fusarium sp. Sa/1/2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal ...
Fusarium oxysporum strain Pc/12/1 internal transcribed spacer 1, partial sequence; 5....
Fusarium oxysporum strain Ks/9/1 internal transcribed spacer 1, partial sequence; 5....
Penicillium minioluteum strain Ks/8/2 internal transcribed spacer 1, partial sequence;...
Fusarium oxysporum strain Af/8/2 internal transcribed spacer 1, partial sequence; 5....
Fusarium oxysporum strain TBCui internal transcribed spacer 1, partial sequence; 5....
Sordariomycetes sp. MD1 internal transcribed spacer 1, partial sequence; 5.8S ribos...
Fungal endophyte strain 1101 18S ribosomal RNA gene, partial sequence; internal tr...
ascomycetes | 3 leaves
ascomycetes | 4 leaves
ascomycetes | 13 leaves
0.005

Bacillus thuringiensis serovar kurstaki strain PTK2G 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|EU588682.1| Length: 789 Number of Matches: 1

Range 1: 26 to 574 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
948 bits(1050)	0.0	539/549(98%)	0/549(0%)	Plus/Plus

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Query  53   CGGGGGACGGGTGAGTACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA  112
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Sbjct  26   CGGCGGACGGGTGAGTACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA  85

Query  113  CCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCG  172
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Sbjct  86   CCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCG  145

Query  173  GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA  232
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Query  233  AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  292
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Sbjct  206  AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  265

Query  293  CCAAACTCCTACGGGAGGCAGCATTAGGGAATCTTCCGCAATGGACGAAAGTCTGANGGA  352
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Sbjct  266  CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  325

Query  353  GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAAAAC  412
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Sbjct  326  GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAAC  385

Query  413  AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTANCCAGAAAGCCACGGCTAACT  472
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Sbjct  386  AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT  445

Query  473  ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGACTTATTGGGCGTA  532
            |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  446  ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA  505

Query  533  AAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGCGAACCCCCACGGCTCAACCGTGGAGGG  592
            |||||||||||||||||||||||||||||||| |||  ||||||||||||||||||||||
Sbjct  506  AAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGG  565

Query  593  TCATTGGAA  601
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Sbjct  566  TCATTGGAA  574
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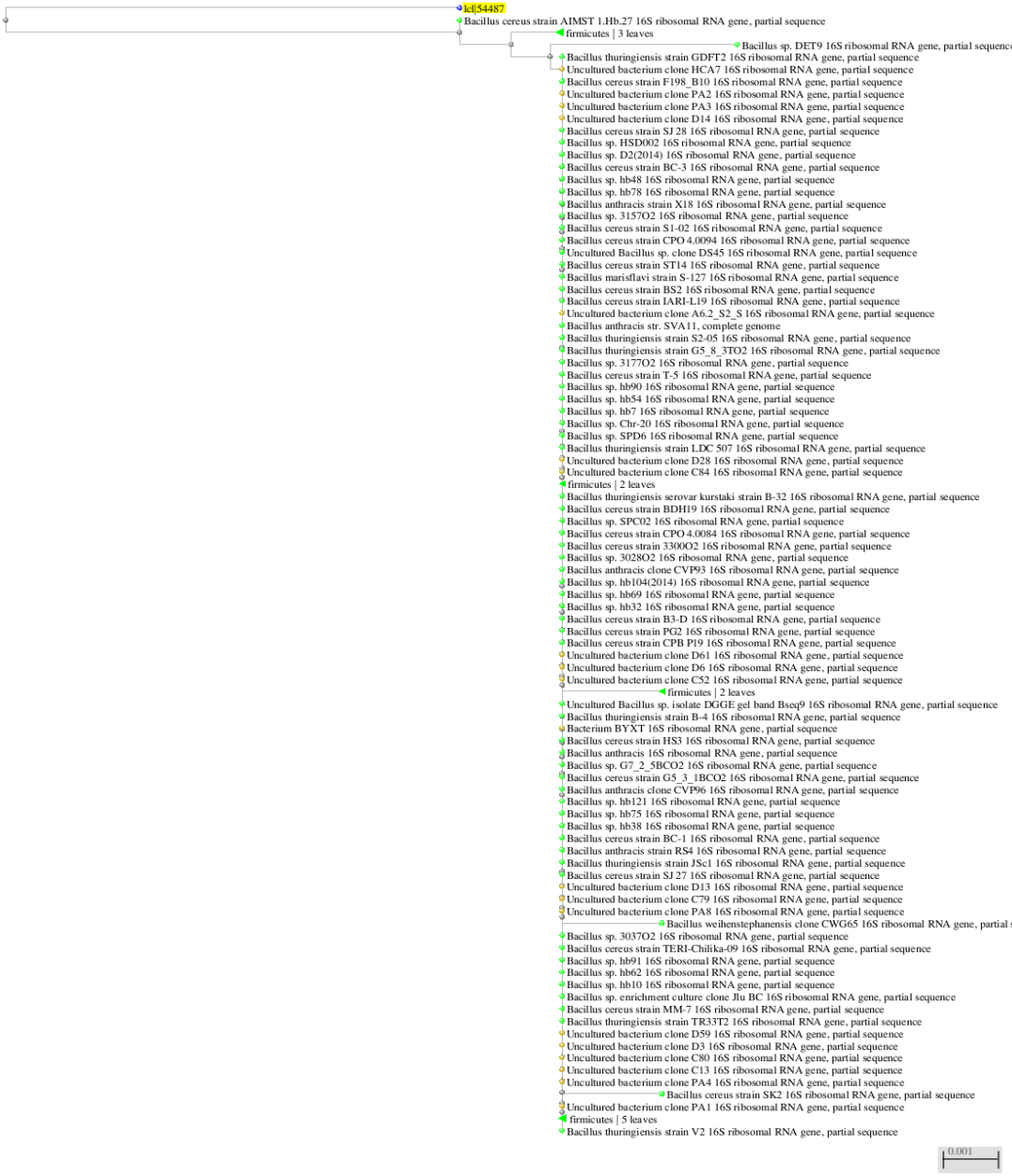
lcl|54487
Bacillus cereus strain AIMST 1.Hb.27 16S ribosomal RNA gene, partial sequence
firmicutes | 3 leaves
Bacillus sp. DET9 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain GDFT2 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone HCA7 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain F198_B10 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone PA2 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone PA3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D14 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain SJ 28 16S ribosomal RNA gene, partial sequence
Bacillus sp. HSD002 16S ribosomal RNA gene, partial sequence
Bacillus sp. D2(2014) 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain BC-3 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb48 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb78 16S ribosomal RNA gene, partial sequence
Bacillus anthracis strain X18 16S ribosomal RNA gene, partial sequence
Bacillus sp. 3157O2 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain S1-02 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain CPO 4.0094 16S ribosomal RNA gene, partial sequence
Uncultured Bacillus sp. clone DS45 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain ST14 16S ribosomal RNA gene, partial sequence
Bacillus marisflavi strain S-127 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain BS2 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain IARI-L19 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone A6.2_S2_S 16S ribosomal RNA gene, partial sequence
Bacillus anthracis str. SVA11, complete genome
Bacillus thuringiensis strain S2-05 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain G5_8_3TO2 16S ribosomal RNA gene, partial sequence
Bacillus sp. 3177O2 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain T-5 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb90 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb54 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb7 16S ribosomal RNA gene, partial sequence
Bacillus sp. Chr-20 16S ribosomal RNA gene, partial sequence
Bacillus sp. SPD6 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain LDC 507 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D28 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C84 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
Bacillus thuringiensis serovar kurstaki strain B-32 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain BDH19 16S ribosomal RNA gene, partial sequence
Bacillus sp. SPC02 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain CPO 4.0084 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain 3300O2 16S ribosomal RNA gene, partial sequence
Bacillus sp. 3028O2 16S ribosomal RNA gene, partial sequence
Bacillus anthracis clone CVP93 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb104(2014) 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb69 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb32 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain B3-D 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain PG2 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain CPB P19 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D61 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D6 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C52 16S ribosomal RNA gene, partial sequence
firmicutes | 2 leaves
Uncultured Bacillus sp. isolate DGGE gel band Bseq9 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain B-4 16S ribosomal RNA gene, partial sequence
Bacterium BYXT 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain HS3 16S ribosomal RNA gene, partial sequence
Bacillus anthracis 16S ribosomal RNA gene, partial sequence
Bacillus sp. G7_2_5BCO2 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain G5_3_1BCO2 16S ribosomal RNA gene, partial sequence
Bacillus anthracis clone CVP96 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb121 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb75 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb38 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain BC-1 16S ribosomal RNA gene, partial sequence
Bacillus anthracis strain RS4 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain JSc1 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain SJ 27 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D13 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C79 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone PA8 16S ribosomal RNA gene, partial sequence
Bacillus weihenstephanensis clone CWG65 16S ribosomal RNA gene, partial sequence
Bacillus sp. 3037O2 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain TERI-Chilika-09 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb91 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb62 16S ribosomal RNA gene, partial sequence
Bacillus sp. hb10 16S ribosomal RNA gene, partial sequence
Bacillus sp. enrichment culture clone Jlu BC 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain MM-7 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain TR33T2 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D59 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C80 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C13 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone PA4 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain SK2 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone PA1 16S ribosomal RNA gene, partial sequence
firmicutes | 5 leaves
Bacillus thuringiensis strain V2 16S ribosomal RNA gene, partial sequence
0.001

Bacillus cereus strain BS2 16S ribosomal RNA gene, partial sequence

Sequence ID: gb|KF672365.1| Length: 1525 Number of Matches: 1

Range 1: 39 to 735 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1238 bits(1372)	0.0	693/697(99%)	1/697(0%)	Plus/Plus

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Query  1    GCCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCG  60
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Sbjct  39   GCCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCG  98

Query  61   GACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  99   GACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGG  158

Query  121  GGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  159  GGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTG  218

Query  181  TCACTTATGGATGGACCCGCGTCGCATTANCTAGTTGGTGAGGTAACGGCTCACCAAGGC  240
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Sbjct  219  TCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC  278

Query  241  AACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG  300
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Sbjct  279  AACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG  338

Query  301  ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAA  360
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Sbjct  339  ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAA  398

Query  361  CGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGT  420
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Sbjct  399  CGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGT  458

Query  421  GCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGT  480
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Sbjct  459  GCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGT  518

Query  481  GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGC  540
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Sbjct  519  GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGC  578

Query  541  GCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCAT  600
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Sbjct  579  GCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCAT  638

Query  601  TGGAAACTGGGAGACTTGAGTGCAGAANAGGAAAGTGGAATTCCATGTGTAGCGGTGAAA  660
            |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  639  TGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAA  698

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            |||||| ||||||||||||| ||||||||||||||||
Sbjct  699  TGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCG  735
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kcl|61773
Bacillus anthracis str. A16, complete genome
Bacillus cereus strain JN19 16S ribosomal RNA gene, partial sequence
Bacillus anthracis strain JN22 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN38 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN66 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN78 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN119 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN155 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN165 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN178 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN234 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN236 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN240 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN259 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain S19 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain BAB-2592 16S ribosomal RNA gene, partial seq...
Bacillus cereus strain IARI-HHS1-17 16S ribosomal RNA gene, partial seque...
Bacillus cereus strain IARI-BHD-3 16S ribosomal RNA gene, partial sequence
Bacillus sp. R23 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain STN-6 16S ribosomal RNA gene, partial sequence
Bacillus cereus partial 16S rRNA gene, strain PrM
Bacillus cereus strain CH-13 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain moh2 16S ribosomal RNA gene, partial sequence
Bacillus sp. LS3 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JF9 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JF16 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JF20 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JF70 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain ASDS9 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain ASK16 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JF14 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain AHBR1 16S ribosomal RNA gene, partial sequence
Bacillus sp. AHBR2 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain AHBR3 16S ribosomal RNA gene, partial sequence
Bacillus thuringiensis strain AHBR14 16S ribosomal RNA gene, partial seque...
Bacillus cereus 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain WG38 16S ribosomal RNA gene, partial sequence
Bacillus anthracis strain I13 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain IHB B 379 16S ribosomal RNA gene, partial sequence
Bacillus anthracis strain IHB B 2660 16S ribosomal RNA gene, partial sequence
Bacterium A7(2013) 16S ribosomal RNA gene, partial sequence
Bacterium A13(2013) 16S ribosomal RNA gene, partial sequence
Bacterium A15(2013) 16S ribosomal RNA gene, partial sequence
Bacterium A18(2013) 16S ribosomal RNA gene, partial sequence
Bacterium A24(2013) 16S ribosomal RNA gene, partial sequence
Bacterium A26(2013) 16S ribosomal RNA gene, partial sequence
Bacterium A28(2013) 16S ribosomal RNA gene, partial sequence
Bacterium A55(2013) 16S ribosomal RNA gene, partial sequence
Bacillus sp. A52 16S ribosomal RNA gene, partial sequence
Bacillus sp. B7 16S ribosomal RNA gene, partial sequence
Bacillus sp. B10 16S ribosomal RNA gene, partial sequence
Bacillus sp. C20 16S ribosomal RNA gene, partial sequence
Bacillus sp. C37-2 16S ribosomal RNA gene, partial sequence
firmicutes | 7 leaves
Bacillus cereus strain BAB-2822 16S ribosomal RNA gene, partial sequence
Bacillus sp. B5 HMK-2013 partial 16S rRNA gene, strain B5
Bacillus cereus strain SBTBC-008 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain SBTBc-001 16S ribosomal RNA gene, partial sequence
Bacillus sp. DmBR 17 16S ribosomal RNA gene, partial sequence
Bacillus sp. DmBR 40 16S ribosomal RNA gene, partial sequence
Bacillus sp. DmBR 22 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain BAB-1323 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain JN244 16S ribosomal RNA gene, partial sequence
Bacillus sp. BSP13 16S ribosomal RNA gene, partial sequence
Bacillus sp. BAB-3111 16S ribosomal RNA gene, partial sequence
Bacillus sp. BAB-3112 16S ribosomal RNA gene, partial sequence
Bacillus sp. BAB-3124 16S ribosomal RNA gene, partial sequence
Bacillus sp. BAB-3128 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone PA3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone PA4 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone PA8 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C13 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C52 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C79 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C80 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone C84 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D3 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D6 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D13 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D14 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D28 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D59 16S ribosomal RNA gene, partial sequence
Uncultured bacterium clone D61 16S ribosomal RNA gene, partial sequence
Bacillus anthracis strain RS4 16S ribosomal RNA gene, partial sequence
Bacillus sp. enrichment culture clone Jlu BC 16S ribosomal RNA gene, parti...
Bacillus sp. hb7 16S ribosomal RNA gene, partial sequence
Bacillus anthracis clone CVP93 16S ribosomal RNA gene, partial sequence
Bacillus anthracis clone CVP96 16S ribosomal RNA gene, partial sequence
Bacillus cereus strain HS3 16S ribosomal RNA gene, partial sequence
Uncultured Bacillus sp. clone DS45 16S ribosomal RNA gene, partial sequence
Bacterium BYXT 16S ribosomal RNA gene, partial sequence
firmicutes | 3 leaves
0.0002

Trichoderma harzianum strain 86 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen

Sequence ID: gb|JX982444.1| Length: 549 Number of Matches: 1

Range 1: 17 to 549 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
962 bits(1066)	0.0	533/533(100%)	0/533(0%)	Plus/Minus

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Query  1    GAGGTCACATTTCAGAAGTTGGGTGTTTAACGGCTGTGGACGCGCCGCGCTCCCGATGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  549  GAGGTCACATTTCAGAAGTTGGGTGTTTAACGGCTGTGGACGCGCCGCGCTCCCGATGCG  490

Query  61   AGTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATTTCGGAGACG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  489  AGTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATTTCGGAGACG  430

Query  121  GCCACCCGCTAAGGGAGGGCCGATCCCCAACGCCGACCCCCCGGAGGGGTTCGAGGGTTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  429  GCCACCCGCTAAGGGAGGGCCGATCCCCAACGCCGACCCCCCGGAGGGGTTCGAGGGTTG  370

Query  181  AAATGACGCTCGGACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  369  AAATGACGCTCGGACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAG  310

Query  241  ATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  309  ATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTC  250

Query  301  ATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATTTTCGAAACGCCTA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  249  ATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATTTTCGAAACGCCTA  190

Query  361  CGAGAGGCGCCGAGAAAGGCTCAGATTATaaaaaaaCCCGCGAGGGGGTATACAATAAGA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  189  CGAGAGGCGCCGAGAAAGGCTCAGATTATAAAAAAACCCGCGAGGGGGTATACAATAAGA  130

Query  421  GTTTTAGGTTGGTCCTCCGGCGGGCGCCTTGGTCCGGGGCTGCGACGCACCCGGGGCAGA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  129  GTTTTAGGTTGGTCCTCCGGCGGGCGCCTTGGTCCGGGGCTGCGACGCACCCGGGGCAGA  70

Query  481  GATCCCGCCGAGGCAACAGTTTGGTAACGTTCACATTGGGTTTGGGAGTTGTA  533
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  69   GATCCCGCCGAGGCAACAGTTTGGTAACGTTCACATTGGGTTTGGGAGTTGTA  17
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Fungal sp. ARIZ B053.p 18S ribosomal RNA gene, partial sequence; internal t...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1094 18S ribos...
ascomycetes | 41 leaves
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1039 18S ribos...
Trichoderma sp. 4 BRO-2013 18S ribosomal RNA gene, partial sequence; inte...
Hypocrea lixii strain SHMH1102 18S ribosomal RNA gene, partial sequence; ...
Hypocrea lixii strain P49P11 18S ribosomal RNA gene, partial sequence; inte...
Trichoderma harzianum strain P134_D1_11 18S ribosomal RNA gene, partial ...
Hypocrea lixii isolate OTU660 internal transcribed spacer 1, partial sequence;...
Trichoderma cf. harzianum ROG-2010 strain IB59b 18S ribosomal RNA gene,...
Hypocrea lixii 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S...
Hypocrea lixii 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S...
Hypocrea lixii strain C.P.K. 1941 internal transcribed spacer 1, 5.8S ribosomal...
Fungal sp. ARIZ B511.p 18S ribosomal RNA gene, partial sequence; internal t...
Fungal sp. ARIZ B511 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B508 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B502 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B474 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B473 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B453 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B447 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B446cA 18S ribosomal RNA gene, partial sequence; internal...
Fungal sp. ARIZ B442 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B440 18S ribosomal RNA gene, partial sequence; internal tr...
Hypocrea lixii strain DAOM 231402 18S ribosomal RNA gene, partial seque...
Fungal sp. ARIZ B423 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B418 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B414cA 18S ribosomal RNA gene, partial sequence; internal...
Fungal sp. ARIZ B408 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B403 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B402cB 18S ribosomal RNA gene, partial sequence; internal...
Fungal sp. ARIZ B402cA 18S ribosomal RNA gene, partial sequence; internal...
Fungal sp. ARIZ B261 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B150 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B149 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B137 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B131 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B104 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B102 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B097 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B094 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B092 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B090 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B089 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B056 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B031 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B007 18S ribosomal RNA gene, partial sequence; internal tr...
Hypocrea lixii strain DIS 221D 18S ribosomal RNA gene, partial sequence; i...
Hypocrea lixii strain GJS 05-101 18S ribosomal RNA gene, partial sequence; ...
Hypocrea lixii strain GJS 05-469 18S ribosomal RNA gene, partial sequence; ...
Hypocrea lixii 5.8S rRNA gene, ITS1 and ITS2, strain VI03700
Hypocrea lixii strain DAOM 229971 18S ribosomal RNA gene, partial seque...
Hypocrea lixii strain JB T1244 18S ribosomal RNA gene, partial sequence; int...
Trichoderma harzianum isolate H-20 internal transcribed spacer 1, partial sequ...
Trichoderma harzianum strain TR274 internal transcribed spacer 1, partial seq...
Trichoderma atroviride strain wxm146 18S ribosomal RNA gene, partial sequ...
Trichoderma atroviride strain wxm143 18S ribosomal RNA gene, partial sequ...
kcl|45585
fungi | 2 leaves
Trichoderma sp. TPK_IPDA5 18S ribosomal RNA gene, partial sequence; inte...
0.0001

Trichoderma tawa strain PS08UC8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1

Sequence ID: gb|KC847172.1| Length: 613 Number of Matches: 1

Range 1: 40 to 612 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1011 bits(1120)	0.0	571/574(99%)	3/574(0%)	Plus/Plus

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Query  3    AACCCA-TGTGA-CGTTACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCG  60
            |||||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  40   AACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCG  99

Query  61   CAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCTAAAACTCTTATTGTATACCCCCT  120
            |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  100  CAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACC-AAAACTCTTATTGTATACCCCCT  158

Query  121  CGCGGGttttttttATAATCTGAGCCTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  159  CGCGGGTTTTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGA  218

Query  181  ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  219  ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATG  278

Query  241  CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  279  CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG  338

Query  301  CCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  339  CCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCC  398

Query  361  GGGGGGTCGGCGTTGGGGATCGGCCCTCCCTTAGCGGGTGGCCGTCTCCGAAATACAGTG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  399  GGGGGGTCGGCGTTGGGGATCGGCCCTCCCTTAGCGGGTGGCCGTCTCCGAAATACAGTG  458

Query  421  GCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACACTCGCATCGGGAGCGCGGCG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  459  GCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACACTCGCATCGGGAGCGCGGCG  518

Query  481  CGTCCACAGCCGTTAAACACCCAACTTCTGAAATGTTGACCTCGGATCAGGTAGGAATAC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  519  CGTCCACAGCCGTTAAACACCCAACTTCTGAAATGTTGACCTCGGATCAGGTAGGAATAC  578

Query  541  CCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  574
            ||||||||||||||||||||||||||||||||||
Sbjct  579  CCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  612
```


ascomycetes | 6 leaves
fungi | 3 leaves
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and ITS2, and 28S ribosomal RNA...
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and ITS2, and 28S r...
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and ITS2, a...
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and IT...
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and I...
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and ...
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and ...
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and ...
Hypocrea lixii genomic DNA containing ITS1, 5.8S ribosomal RNA gene and ...
Hypocrea nigricans genomic DNA containing ITS1, 5.8S ribosomal RNA ge...
Hypocrea lixii strain SzMC 3203 internal transcribed spacer 1, partial sequen...
Trichoderma sp. 6 BRO-2013 18S ribosomal RNA gene, partial sequence; inte...
Trichoderma sp. 7 BRO-2013 18S ribosomal RNA gene, partial sequence; inte...
Trichoderma sp. HYKH1/A2 18S ribosomal RNA gene, partial sequence; inte...
Trichoderma sp. HYKH2/B2 18S ribosomal RNA gene, partial sequence; inte...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1094 18S ribos...
Trichoderma sp. 4 BRO-2013 18S ribosomal RNA gene, partial sequence; inte...
Fungal sp. ARIZ B508 18S ribosomal RNA gene, partial sequence; internal tr...
Hypocrea lixii strain DAOM 231402 18S ribosomal RNA gene, partial sequen...
Fungal sp. ARIZ B403 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B007 18S ribosomal RNA gene, partial sequence; internal tr...
Hypocrea lixii genomic DNA containing ITS1, 5.8S rRNA gene and ITS2, stra...
Hypocrea sp. 3 TMS-2011 voucher MS3p_50-23 18S ribosomal RNA gene, p...
Hypocrea lixii strain IMI 359823 internal transcribed spacer 1, partial sequen...
Trichoderma sp. HYKH4/C1 18S ribosomal RNA gene, partial sequence; inte...
Trichoderma sp. HYKH3/C1 18S ribosomal RNA gene, partial sequence; inte...
kel|30101
ascomycetes | 3 leaves
Trichoderma sp. HYKHK/B2 18S ribosomal RNA gene, partial sequence; inte...
Trichoderma harzianum strain TUB F-834 internal transcribed spacer 1, parti...
Hypocrea lixii strain TUB F-444 internal transcribed spacer 1, partial sequen...
Hypocrea lixii strain TUB F-961 internal transcribed spacer 1, partial sequen...
Hypocrea lixii genomic DNA containing ITS1, 5.8S rRNA gene and ITS2, stra...
Hypocrea lixii isolate C40065P301CC594 internal transcribed spacer 1, 5.8S r...
Trichoderma harzianum strain T-alces internal transcribed spacer 1, partial seq...
Trichoderma harzianum strain T-soybean internal transcribed spacer 1, partial ...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1215 18S ribos...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1024 18S ribos...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1010 18S ribos...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1161 18S ribos...
Hypocrea nigricans strain NBRC 30611 internal transcribed spacer 1, partial s...
Hypocrea lixii strain JB T2276 18S ribosomal RNA gene, partial sequence; int...
Hypocrea lixii strain JB T2181 18S ribosomal RNA gene, partial sequence; int...
Hypocrea lixii strain DAOM 231646 18S ribosomal RNA gene, partial sequen...
Trichoderma harzianum strain TUB F-1035 internal transcribed spacer 1, part...
Hypocrea lixii strain CBS 115337 internal transcribed spacer 1, partial sequen...
Hypocrea lixii isolate B1A0031EM2CC1133 internal transcribed spacer 1, 5....
Fungal endophyte strain MS192 18S ribosomal RNA gene, internal transcribed...
Trichoderma tawa strain PS08UC8 18S ribosomal RNA gene, partial sequenc...
Hypocrea lixii strain DAOM 231408 18S ribosomal RNA gene, partial sequen...
Hypocrea lixii isolate MIAE00042 18S ribosomal RNA gene, partial sequenc...
Trichoderma harzianum strain TUB F-1078 internal transcribed spacer 1, part...
Hypocrea lixii isolate B1a0034EM2CC39 internal transcribed spacer 1, 5.8S r...
Fungal endophyte strain MS153 18S ribosomal RNA gene, internal transcribed...
Fungal endophyte strain MS248 18S ribosomal RNA gene, internal transcribed...
Fungal endophyte strain MS380 18S ribosomal RNA gene, internal transcribed...
Fungal endophyte strain MS429 18S ribosomal RNA gene, internal transcribed...
Hypocrea sp. PALM1 internal transcribed spacer 1, partial sequence; 5.8S ribo...
Hypocrea lixii strain JB T1244 18S ribosomal RNA gene, partial sequence; int...
Hypocrea lixii strain DAOM 229971 18S ribosomal RNA gene, partial sequen...
Fungal sp. ARIZ B104 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B418 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B423 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B453 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal endophyte culture-collection STRI:ICBG-Panama:TK1039 18S ribos...
Hypocrea lixii isolate B1aA1SNA1CC43 internal transcribed spacer 1, 5.8S r...
Hypocrea lixii strain DAOM 230830 18S ribosomal RNA gene, partial sequen...
Hypocrea lixii strain Tri102 18S ribosomal RNA gene, partial sequence; inter...
Fungal sp. ARIZ B031 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B089 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B092 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B094 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B097 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B137 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B261 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B402cA 18S ribosomal RNA gene, partial sequence; internal...
Fungal sp. ARIZ B402cB 18S ribosomal RNA gene, partial sequence; internal...
Fungal sp. ARIZ B408 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B414cA 18S ribosomal RNA gene, partial sequence; internal...
Fungal sp. ARIZ B440 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B442 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B446cA 18S ribosomal RNA gene, partial sequence; internal...
Fungal sp. ARIZ B473 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B502 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B511 18S ribosomal RNA gene, partial sequence; internal tr...
Trichoderma atroviride isolate UOKT045 small subunit ribosomal RNA gene,...
Fungal sp. ARIZ B447 18S ribosomal RNA gene, partial sequence; internal tr...
Fungal sp. ARIZ B474 18S ribosomal RNA gene, partial sequence; internal tr...
ascomycetes | 2 leaves
0.0002