The Environmental Role of Yeasts-Emphasis on Occurrence and Survival in the Built Environment Including Health-Care Settings

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In the name of Allah most gracious most merciful

and

Praise be to God

Dedication

I am pleased to dedicate my thesis to those who inspired me to the higher ideals of life my beloved parents and fabulous family particularly my wonderful wife Dukhnah also to beloved four sons Yazan, Faris, Iyad and Najjad, With them, I completed this thesis with their continuous and sincere advice, and full support, and encouragement throughout the period of my study, and to my brothers and sisters in Saudi Arabia.

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ABSTRACT

A study was made of the distribution of yeast in the environment, notably the indoor environment relative to health care situations. The Thesis begins with a description of work aimed at isolating yeasts from an inorganic soil and from bovine dung. Three species of *Candida* were isolated from the latter, but no yeasts were found in the mineral, agricultural soil tested. The two *Candida* isolates with 100% matches to *Candida* sp. were shown to hydrolyze urea, oxidize ammonium to nitrate, solubilise elemental sulfur and solubilise a source on insoluble phosphate. The focus of the Thesis then moves to the isolation of yeasts from the indoor environment. A wide range of yeast and filamentous fungi were isolated from computer keyboards, sinks, used tooth brushes and vacuum cleaner dust. Yeasts were shown to be emitted by hand dryers, the use of which is becoming increasingly popular. It is suggested paper towels be used in preference to these machines in health care settings, as hand dryers can spread potential pathogens into the air. Studies were also made on the ability of yeasts to survive on smooth and rough tiles similar to those found in homes and hospitals. Paradoxically, yeasts were shown to survive longer on smooth, compared to rough tiles and on plastic plumbing fittings than on copper fittings; in fact copper fittings appear ideal as they were seen to dramatically reduce the survival of potentially pathogenic yeasts and, as a result, should be used in preference to plastic piping for use in hospitals and other healthcare settings.

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CHAPTER 1

1.1. Introduction.

A wide range of microorganisms exist in the environment, including viruses, bacteria, yeasts and filamentous fungi. Fungi are causal agents of many plant and animal diseases although their role as human pathogens is often underplayed. In a recent study setting (Perlroth *et al*., 2007)., fungi in general were estimated to kill at least as many people as tuberculosis or malaria. Over the past decades, yeast infections have become a major problem to be overcome and yeasts contain several species that cause superficial as well as invasive systemic diseases in humans and other animals. Their pathogenicity is particularly dangerous in the case of immunocompromised patients, in which they cause potentially life-threatening diseases (Sullivan *et al.*, 1997). The most important disease causing yeasts are members of the genus *Candida*. Among *Candida* sp., *C. albicans* and *C. glabrata* are ranked as the first and second in terms of isolation and incidence respectively and together are accountable for about 65%–75% of all systemic candidiasis, followed by *C. parapsilosis* and *C. tropicalis* (Perlroth *et al*., 2007). Currently, *C. albicans* is the most well studied yeast species, largely because of its clinical significance as a major causal agent of mucosal and systemic infections, notably of AIDS patients. A range of different species of *Candida* sp. have been isolated routinely from humans (Kurtzman and Robnett, 1998). Although yeast are primarily considered as human and animal pathogens, or known for their role in food biotechnology (e.g. in baking and brewing), they also play an, increasingly recognized, major role in the environment, notably water, soil, leaves and flowers and the dung of cattle, chickens and humans, usually as a non-pathogenic resident. Kirk and Bartlett (1986) and Okamoto *et al*. (1988) isolated some *Candida* sp. from milk samples taken from cows suffering from mastitis. Wyatt *et al*. (1975) showed the incidence of *Candida* in the crops to be

approximately [100%], and also in healthy birds, while Williams *et al.* (1977) showed that *Candida* can be an important aetiological agent of abortion in cattle. Clark (1960) found that bovine mycotic (i.e. yeast) mastitis may occur by infection via licking or suckling of the udder quarters by lactating calves or through contamination of the udder with the animal's own dung. Yeasts have also been isolated regularly from the gut and surface of insects that feed on a variety of materials, including Basidiomycete fruiting bodies, woody substrates, ephemeral flowers and nectar exudates (Kurtzman *et al*., 2001; Marinoni and Lachance, 2004). Plants and insects are also frequently associated with yeasts (Suh *et al*., 2006) and in most cases, yeast clades, or metabolic guilds, have specific associations with certain insects (Suh *et al*., 2003). Some *Candida* sp. can also form biofilms on the surfaces of both organic and inorganic materials. Relatively little is known about the role of yeasts in the environment and the factors which influence their survival. This relative lack of information is potentially damaging since, as we have seen, many yeasts (notably species of *Candida*) act as animal and human pathogens, especially in immunocompromised patients. The work described here begins with a discussion of the isolation of yeasts from cow dung and a study of the role of some of these isolates in various component parts of the major biogeochemical cycles, i.e. of N, the S and P cycles. The work continues to concentrate on the role of yeasts in the environment but undergoes a major shift in emphasis away from the agricultural setting, to concentrate on studies on isolation of yeasts from the built environment and to the determination of some of the factors with influence their survival in these habits. The emphasis here is redirected to the presence and survival of yeasts, notably *Candida species*, in relation to hospitals, and other health care settings.

1.2. Molecular biology techniques.

A molecular approach, namely 18S rRNA was used to identify the yeasts isolates studied here. It is possible to isolate deoxyribonucleic acid (DNA) from many different specimens. Recently, many techniques are available for extract DNA from many different sources such as tissues, blood, bones, sperm, plant, hair and bacteria (Lahiri, 1992). Isolated DNA is used in many different studies, e.g. for diagnostic tests in laboratory and for criminal investigation. Molecular biology techniques involve the identification of a microorganism and its characteristics by molecular techniques which require a gene sequence and understanding the interactions between the various systems of a cell, including the interactions between the different types of DNA, RNA and protein biosynthesis. DNA analysis has also been applied at different resolution levels for fungal, bacterial and yeast isolates and clones of specific genes (Hill *et al.,* 2000). Several methods are available now to isolate DNA from many different specimens. Firstly, tissues or cells are broken then the cells are lysed by using enzymes or detergents then centrifuged to separate the DNA from other components followed by purification from other molecules (Amann *et al.,* 1995).

1.3. Deoxyribonucleic acid (DNA).

DNA controls the genetic instructions used in the functioning and development of living organisms and it is the major macromolecular essential for all known living organisms. It contains the information needed to construct other cell components such as RNA molecules and proteins (Van Holde, 1989). DNA is a double stranded and contains four chemical bases, which are adenine (A), thymine (T), cytosine (C) and guanine (G) (Baker *et al.,* 2006). To form units called base pairs, A only binds with T and C with G (Maier *et al.,* 2009) (Figure1.1).

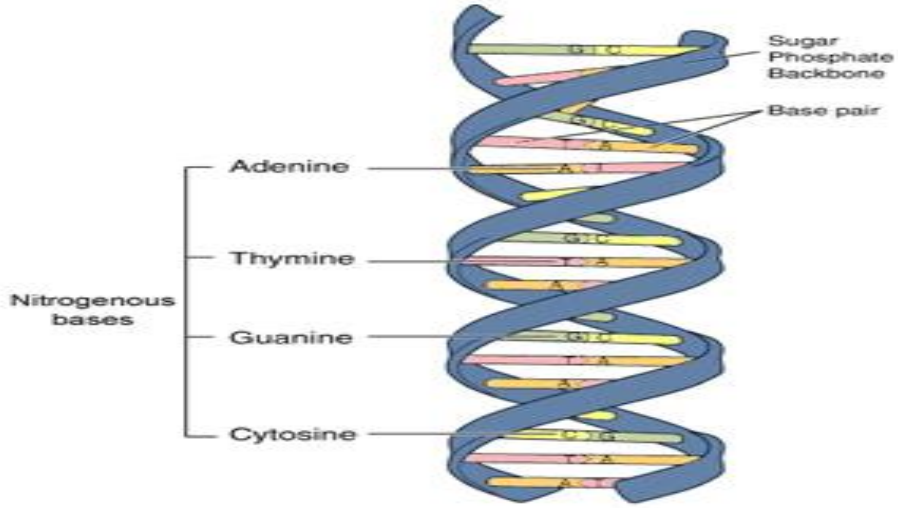


Figure 1.1: Showing the DNA double helix and base pairs (Image adapted from: National Human Genome Research Institute).

DNA consisting of two polymers made of units called nucleotides, which form a backbone of DNA the alternating sugar and phosphate groups in each strand of DNA also confer directionality one backbone is oriented 5`to 3`while the complementary strand is oriented 3`to 5` (Madigan *et al.*, 2012).

1.4. Polymerase chain reaction (PCR) technique.

PCR is a molecular technique used in the laboratories of medical and biological research to exponentially amplify particular DNA sequences within a few hours (Hadidi and Candresse, 2003). The PCR technique is a molecular method for amplifying target gene and allowing access to the genomic information from non-culturable microorganisms also it is sensitive method for detection of nucleic acids, disease identification and to the discovery of pathogens (Hadidi and Candresse, 2003; Maier *et al.*, 2009). There are three main stages of the PCR, these being repeated for a number of cycles to exponentially increase the number of copies of a specific target region (Henson and French, 1993) (Figure 1.2).

Stage 1 denaturation (melting of target DNA), involves the denaturation of the double stranded DNA into two single strands of template DNA by heating the DNA to 94^{o}C during

20 to 40 seconds. Stage 2 annealing of primers (annealing of two oligonucleotide primers to the denatured DNA strands), involves the lowered the temperature around 50 to 70°C during 20 to 40 seconds to allow the primers to bind. Stage 3 involves the extension by a polymerase (primer extension by a thermo-stable DNA polymerase) involves the incorporation of (dNTPs; A, C, G, T), thereby extending the DNA sequence in the 5`to 3`directions by raised the temperature (72°C - 74°C) which depends on the DNA polymerase used.

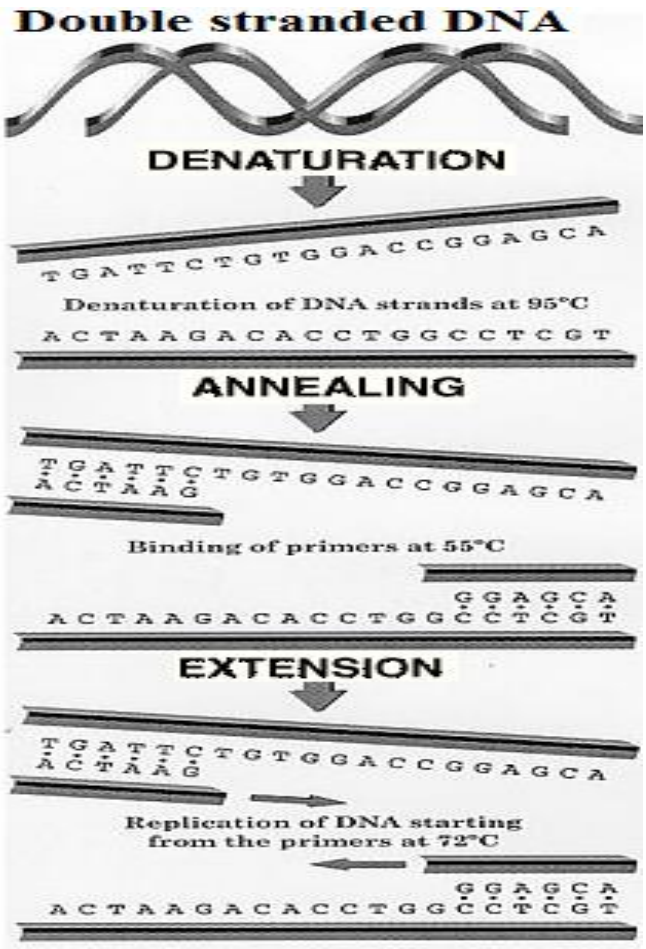


Figure 1.2: The polymerase chain reaction (PCR) cycle (Mullis, 1995).

1.5. Aims of the work described in this Thesis.

The aim of the work described here was to:

1) Determine yeast diversity in cow dung and soil using the molecular identification technique, 18S rRNA gene sequencing, and determine the potential role of these isolates in the major biogeochemical cycles.

2) Determine the factors which influence the survival of *Candida* in the built environment (e.g. on ceramic tiles, on sinks, on used toothbrushes etc, notably in relation to the survival of pathogenic yeast in hospitals and other medical situations.

CHAPTER 2

STUDIES ON YEAST DIVERSITY IN FRESH COW DUNG AND A SOIL SAMPLE USING 18S RNA GENE SEQUENCING.

2.1. Introduction.

The part played by yeasts in the major biogeochemical cycles in soils and waters has been generally overlooked, certainly in comparison to the vast numbers of papers published on the role of filamentous fungi and bacteria in these environments. It is well–recognized that microorganisms play a key role in the functioning of the environment in relation to the biogeochemical cycles and they also play crucial roles in the transformations and recycling of essential elements; they can also result in unique contributions to biogeochemical cycles and biotechnological processes. This Chapter describes a study which was made of the isolation of yeasts from cattle dung and the potential role of these organisms in aspects of the nitrogen cycle (the hydrolysis of urea to ammonium and oxidation of ammonium to nitrate), the sulphur cycle (the oxidation of elemental sulphur) and the phosphorus cycle (the solubilisation of a form of insoluble phosphate).

2.2. The Nitrogen Cycle.

Nitrogen is a major component of amino acids which are the building blocks of peptides and protein, it is also essential for growth and reproduction in both plants and animals and the N-cycle is considered the most important biogeochemical cycle (Pidwirny, 2006). It is part of the genetic material of cells, the nucleic acids and comprises about 80% of the Earth's atmosphere and makes up about 12% of cell dry weight (Maier *et al*, 2009).

Table 2.1: The oxidation states of nitrogen.

Oxidation state	Species	Name
-3	NH_3, NH_4^+	Ammonia, ammonium ion
-2	N_2H_4	Hydrazine
-1	NH_2OH	Hydroxylamine
0	N_2	Nitrogen gas
+1	N_2O	Nitrous oxide
+2	NO	Nitric oxide
+3	HNO_2, NO_2^-	Nitrous acid, nitrite ion
+4	NO_2	Nitrogen dioxide
+5	HNO_3,NO_3^-	Nitric acid, nitrate ion

There are five main processes operating in the N-cycle (Harrison, 2003):

1) Nitrogen fixation: by which atmospheric nitrogen (N_2) is converted to ammonia (NH_3).

2) Nitrogen uptake (organismal growth or assimilation): where microorganisms make use of ammonium to produce organic nitrogen compounds.

3) Nitrogen mineralisation (decay): which organic nitrogen is converted to inorganic nitrogen (ammonium NH_4^+).

4) Nitrification: ammonium (NH_4^+) is oxidized to nitrate (NO_3^-).

5) Denitrification: the reduction of nitrate (NO_3^-) and nitrite (NO_2^-), to nitrous oxide (N_2O), then to a nitrogen gas into the atmosphere.

2.3. Ammonification.

During the processes of decomposition the nitrogen in proteins is transformed to ammonia (NH_3) or ammonium (NH_4) by a variety of microorganisms (bacteria and fungi). This brings

about the release of N from the organic matter present in dead plants and dead animals or dung (Hart *et al.,* 1994).

2.4. Nitrification.

Nitrification is of major importance for the N-cycle in aquatic and terrestrial environments, it involves the oxidation of ammonia (NH_4^+) to nitrite (NO_2^-) and then nitrite to nitrate (NO_3^-) by chemoautotrophic bacteria and by some heterotrophic fungi and bacteria which can also perform these oxidations (Maier *et al.,* 2009). Two types of nitrification exist (Killham, 1994): The first involves the activity of chemoautotrophic nitrifying bacteria (*Nitrosomonas*) by which ammonia (NH_3) or ammonium (NH_4^+) ions are oxidised to nitrite (NO_2^-).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H+ + H_2O$$

The second, involves chemoautotrophic Gram-negative bacteria which oxidize nitrite (NO_2^-) is oxidized to nitrate (NO_3^-) *Nitrobacter.*

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

2.5. Urea hydrolysis.

Urea is highly soluble in water and also has high nitrogen content which exceeds that of ammonium, nitrate and ammonium sulphate (Ferguson *et al.,* 1984). Ureases are enzymes secreted by microorganisms in soil, plants and animals.

Urea is converted to carbon dioxide and ammonia by urease in soil by many microorganisms which are capable of hydrolysing urea such as bacteria, notably species of *Pseudomonas, Achromobacter, Bacillus, Micrococcus* and some fungi notably species of *Penicillium* and most other *Deuteromycetes* (Maier *et al*., 2009).

2.6. The Sulphur cycle.

Sulphur is ranked as the tenth most abundant element in the earth's crust, and is an essential element for growth of all organisms being an essential element for the synthesis of the amino acids, cysteine and methionine, and vitamins such as vitamin B1 (thiamine), hormones such as biotin, coenzymes and lipoid acid (Maier *et al*, 2009). The S-cycle can be summarized as the mineralization of organic sulphur to inorganic sulphate, the oxidation of reduced, inorganic forms to sulphate, the anaerobic reduction of sulphate to sulphides, and the immobilisation of sulphate as organic sulphur (Waksman, 1927). Research shows that filamentous fungi play a role in the S- cycle; *Fusarium solani* (a soil fungus) for example, oxidizes S° to S_2O^{32-}, S_4O^{62-}, and SO^{42} (Wainwright, 1984; Wainwright and Killham, 1980). Fungi oxidize sulphur to sulphate with the formation of tetrathionate and thiosulphate. These products, it has been suggested, may protect fungi from the toxic effects of heavy metals (Wainwright *et al.*, 1997).

Several factors affect sulphur oxidation in environment, including:

1. pH: sulphur oxidation can take place between pH 2 and 9 and sulphur oxidation increases with increasing pH (Vitolins and Swaby, 1969).
2. Temperature: the optimum temperature for S-oxidation range is between 25°C to 40°C, while some thermophilic bacteria and fungi can also grow at 55°C (Wainwright, 1984).
3. Microbial composition: S-oxidation is influenced by the size and composition of the soil microbial community (Soomro, 2000).
4. Moisture and aeration: the moisture content for rapid sulphur oxidation is near field capacity (Mahfouz, 2005).

2.7. The Phosphorus Cycle.

Phosphorus is an essential component of all living organisms and its cycling is second in terms of importance after the nitrogen cycle (Goldstein, 1994). It is a critical element because of its central role in many important biomolecules, notably adenosine triphosphate (ATP), in cell development, in (DNA) deoxyribonucleic acid and in phospholipids (Hyland *et al.*, 2005). Bacteria as well as some species of actinomycetes and fungi can solubilise phosphate (Hattori, 1973; Paul and Clark, 1996). These microorganisms release P when growing in a medium containing calcium phosphate, apatite or similar insoluble source of phosphate; phosphate solubilizing fungi include species of *Aspergillus, Fusarium, Penicillium* and *Sclerotium* (Al-Turk, 1990). Microorganisms are involved in the transformations phosphorus into available sources in a number of ways as follows:

1) Altering the solubility of inorganic P compounds.

2) The mineralization of organic compounds to form inorganic phosphorus.

3) The immobilisation of inorganic phosphorus into cell components.

2.8. Isolation of yeasts from a soil and bovine dung.

Relatively few studies have been done on the isolation of yeasts from soils and other environmental samples. The scarcity of information on yeast in the environment is particularly noticeable compared with the vast amount of information on other microbes in soils and elsewhere. Wyatt *et al.* (1975) however, showed the incidence of *Candida* in the crops of healthy birds ranged from 17.4% to 51.5% with a mean value of 32%. Why yeasts have been neglected in this way is not immediately apparent, but it may relate to the fact that they are usually associated with high carbon (Kirk and Bartlett, 1986) environments and are therefore infrequently present in most agricultural soils and therefore do not play a role in the major biogeochemical cycles. The work described in this Thesis began with an attempt to isolate yeasts from local agricultural soil and bovine dung an offshoot of which was an

opportunity to test the selectivity of the Candida Isolation medium, the means by which isolates were obtained. In the event, no yeasts were isolated from the soil sample used. It was expected however, that the bovine dung prove a better source of yeast isolates, based on the generally accepted view that yeasts prefer organic rich environments, and are isolated using carbon-rich media. Yeasts are known to be associated with cows, so their carbon-rich dung seemed a likely possible source of these organisms; for example, Okamoto *et al*. (1988) isolated species of *Candida* from milk samples taken from healthy cows and those showing signs of mastitis. Williams *et al.* (1977) also isolated *Candida sp*. as the a etiological agent of abortion in cattle and Clark (1960) found that bovine mycotic mastitis may occur by infection via licking or suckling of the udder quarters by lactating calves or through contamination of the udder with the animal's own dung. The role played by yeasts in bovine mastitis provided an additional reason for attempting to isolate yeasts from bovine waste as such work might lead to research on the role of yeasts in animal disease. Once yeasts were isolated then work could commence determining their role in various pathways of the major geochemical cycles. This approach was based on the fact that any yeasts found in cow dung could would likely be passed to soils and there play a potential role in soil processes. Alternatively, mineral transformation could occur within the dung and any products could be released for use by soil microbes and plants.

2.9. Aims of the work described in this Chapter.

The aim of the first part of this project was to determine the yeast diversity (using molecular identification techniques 18S rRNA gene sequencing) in a soil sample and from fresh cow dung. Both classical microbiology and molecular techniques were employed to identify and characterize fungi isolated from several environments. The potential role of the isolates in the major biogeochemical cycles was then determined.

2.10. Materials and Methods.

2.10.1. Sample collection.

A fresh agricultural soil (previous crop potatoes) was obtained from Fitzwilliam, West York's, while cow dung samples were collected from a cattle farm in South Yorkshire (Location given in Figure 2.1).

Figure 2.1: Shows the sampling site used for the collection of cow dung.

2.10.2. Measurement of pH of samples.

The pH of the fresh cow dung and the pH of the soil was determined by using a pH meter fitted with a glass electrode (3310, Jenway Ltd, UK) on soil and cow dung samples (1:1, 10 g of sample in 10 ml distilled water ddH_2O) shaken for 30 minutes as follows: the agricultural soil was pH 7.5 and cow dung pH 8.37.

2.10.3. Isolation of fungi from fresh cow dung and soil.

Fresh cow dung or soil sample (an agricultural loam, previous crop potatoes) (1g) was dissolved in 9 ml of sterilized water into falcon tube 15 ml then mix it by vortex and distributed 100 µl of mixture in each petri dishes containing Candida Ident Agar CIA (Composition from: Peptic digest of animal tissue, Yeast extract, Malt extract, Glucose,

Chloramphenicol, Chromogenic mixture, Agar and pH 7.2 +/- 0.2 (at25^{o}C)) (Table 2.2) The plates were then incubated at 25 °C for 3 to 5 days.

Table 2.2: Composition CIA medium (pH 7.2 +/- 0.2).

Components	**Amount**	**Comments**
CIA	21.5 g	Suspend 21.5g in 500 ml D.W
distilled water dH_2O	500 ml	
Heated in a water bath	90 °C	Boil to dissolve the medium completely onto 90 °C
Candida selective supplement (Gentamicin 50 mg)	1 vials	Medium was dissolved in water Bath onto 90 °C then cool to 50 °C and added 1 vials/l Candida Selective Supplement and pour into sterile Petri plates.

2.10.4. Extraction of genomic DNA by using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit).

Genomic DNA was extracted from each strain of yeast species in cow dung samples and soil samples after inoculation onto Potato Dextrose Broth medium (PDB). PDB is used for the cultivation of fungi, the low pH (pH: 5.1 ± 0.2) for this medium inhibits bacterial growth and encourage luxuriant yeast growth (Table 2.3) (MacFaddin, 1985). Then PCR was used with use of appropriate primers to produce sufficient quantities of 18S rRNA gene.

Table 2.3: Composition Potato Dextrose Broth medium.

Components	**Amount**	**Comments**
Potato Dextrose Broth (PDB)	12 g	
Distilled water dH**2**O	500 ml	Medium was dissolved after mixing by Vortex and then was autoclaved at 120°C for 30 min.

Pure yeast culture (5 ml) grown for 3 to 5 days in Potato Dextrose Broth media were collected and centrifuged by centrifugation at (14,000 rpm) for 1 minute to pellet the cells. The supernatant was then decanted carefully, then the procedure was as follows:

Lysis solution: Re-suspended pellet by adding 500 µl of lysis solution and the cells were re-suspended by gentle vortexing (optional RNase treatment) 10 K Units of RNase was added to the suspension and mixed completely.

Mixture homogeneity: The mixture transferred to a provided (Bead Tube) and vortex horizontally for 5 minutes on a flatbed vortex pad with tape then the mixture were incubated at 65°C for 10 minutes and in during incubation mix the mixture 2 or 3 times.

Centrifugation: The mixture was transferred into a DNase-free micro-centrifuge tube and centrifuge it at (14,000 rpm) for 2 minutes. The supernatant was transferred carefully to a new micro-centrifuge tube.

Addition of ethanol: Equal volume of absolute ethanol (96%-100%) was added to the mixture and vortexed immediately then 300 µl of binding solution was added into the mixture and vortex to mix.

Binding nucleic acids to column: 650 µl of the mixture with ethanol was transferred into a column then centrifuge it at (8,000 rpm) for 1 minute while the flow was discarded and this step was repeated with remaining mixture.

Column wash: 500µl of wash mixture 500 µl of wash solution was washed the column and centrifuge at (8,000 rpm) for 1 minute and discard flow through (wash buffer was diluted by absolute ethanol before use). The column was centrifuged at (8,000 rpm) for 1 minute again to removed residual ethanol. The samples were centrifuged at (14,000 rpm) for 2 minutes and discard the collection tube.

Nucleic Acid elution: A fresh 1.7 ml Elution tube provided with the kit was used to collecting DNA by adding 100 µl of Elution Buffer directly into column membrane then centrifuge at (8,000rpm) for 2 minutes to elute DNA.

Storage of DNA: DNA was stored at -20°C for a few days or -70°C for long-term storage.

2.10.5. Test samples preparation for PCR.

A quantity of 10µl of mixture (samples) with 2 µl loading dye were added to the wells in Agarose gel electrophoresis and to determine the size of fragments, 6 µl of Hyper Ladder was used to make sure of the existence of DNA in the samples.

2.10.6. DNA quantification.

There are several methods for examining DNA quantification. The common one is using a spectrophotometer (Haque *et al*., 2003). 98.0 µl of elution buffer (EB) was added to 2.0 µl of the genomic DNA sample, mixing and filling in special UV cuvettes (UVette, eppendorf) and the optical density OD was measured at 260nm using spectrophotometer (Unicam, Hexios). Amount of DNA was calculated by following this equation:

100÷ 2 = 50 dilution

OD260 × 50 dilution factor = amount of DNA µg/ml.

2.10.7. Polymerase chain reaction (PCR).

The Polymerase Chain Reaction (PCR) was used for amplifying specific gene 18SrRNA after successful extraction of genomic DNA from unknown microorganisms by using 18SrRNA. Universal yeast primers (**Forward**, ITS1: 5` TCCGTAGGTGAACCTGCGG 3`, Length 19 and **Reverse**, ITS4: 5`TCCTCCGCTTATTGATATGC 3`, Length 20) (Simon *et al.,* 1993), that was used for DNA amplification (Reeb *et al.,* 2010) and (Table 2.4) indicates the amount of components of the PCR reaction that were used for amplification of 18S rRNA genes.

Table 2.4: Components of the PCR reaction that were used for amplification of 18S rRNA genes.

Component	**Quantity**
Sterile water	28 µl
10x buffer	5 µl
50mM $MgCl_2$	2.5 µl
Primer **ITS1** (Forward)	4 µl
Primer **ITS4** (Reverse)	4 µl
dNTPs	1 µl
Genomic DNA	5 µl
Bioline Taq	0.5 µl

Initial denaturation was at 98°C for 3 min, followed by DNA denaturation at 94°C for 1 min, primer annealing at 58°C for 1 min, strand elongation at 72°C for 5 min. Final elongation at 75°C for 5 min. The thermal cycling order which was used for amplification of 18S rRNA gene is presented in (Table 2.5).

Table 2.5: The thermal cycling protocol and steps which was used for amplification of 18S rRNA gene.

Steps	**Time**	**Temperature for yeast PCR**	**Number of cycle**
Initialization (Initial denature)	3 min.	98 °C	1
Denature	1 min.	94 °C	35
Annealing	1 min.	58 °C	
Extension/Elongation	5 min.	72 °C	
Final elongation	5 min	75 °C	1
Hold		4 °C	

2.10.8. Agarose gel electrophoresis.

Separating of DNA fragments was achieved after finishing the amplification processes of 18S rRNA genes by using 1% agarose gel. This is achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field. These gels will be prepared in the following manner: Molecular Biology Grade Agarose (0.5 g) was dissolved in 50 ml of Tris-Acetate-EDTA, 1× buffer (pH 8.3 ± 0.1) by heating in a microwave on a medium high power for approximately 3 minutes until the agrarose was dissolved and the solution was cooled and then 2.5 µl Ethidium bromide solution was added after mixing to visualise the DNA before setting the solution in gel tray, followed by pouring the gel in the gel rack. The comb was inserted at one side of the gel and left at room temperature for 30 minutes. Then the comb and seal were removed. The gel was placed into electrophoresis tank immersed in Tris-Acetate-EDTA 1×. The samples of 10 µl after finishing the amplification processes mixed with 2 µl loading dye were added to the wells. In order to determine the size of fragments, 6 µl of Hyper Ladder was used. The samples then underwent electrophoresis for 40 min operated at 85V. The DNA was visualized on the gel and digital image was taken using UV transilluminator attached to a digital camera.

2.10.9. Phylogenetic analysis.

All samples were sent to the University of Sheffield Medical School Core Genetics Unit for further sequencing. BLAST was used to compare the phylogenetic determination of 18S rRNA genes sequences provided from the website of National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov).

2.10.10. Identification of *Candida* and other Yeasts using 18s rRNA gene sequencing.

As discussed by Prosser (2002) and Kirk *et al.* (2004), techniques based on 18SrRNA analyses have been extensively used to identify fungi, despite the fact that the available databases are not as extensive as those available for prokaryotes; relatively few fungi having

been identified from natural environments using these methods (Hill *et al.,* 2000). In order to determine the presence of microbes in soils and other environments both, in terms of numbers or metabolic activity, microbiologists continue to use direct isolation methods as a useful tool, even if they recognize that the organism isolated may not represent the dominant species. 18SrRNA can be successfully employed to identify fungi which have been isolated using traditional methods from, for example, soils (Amann *et al.,* 1997; Scow *et al.,* 2001; Hill *et al.,* 2000) and considerable attention has been given to methods which can be employed to disrupt yeast biomass so as to achieve the best preparations for molecular analysis (Prosser, 2002; Kirk *et al.,* 2004).

2.11. Results.

Candida species were not isolated from the soil samples used. This may have been due to the pH being alkaline or more likely because of low organic content of the soil (which was visually obvious). Isolates of this yeast were however, obtained from cow dung using CIA, This is a selective identification Agar, which facilitates the rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* sp. Using this media results are obtained within 48 hours, showing that this medium is useful for rapid and presumptive identification of common yeasts in both the Mycology and Clinical Microbiology Laboratory (Perry, 1987), (Rousselle *et al.,* 1994). However, it was soon obvious that this medium also isolates filamentous fungi and, as a result in the isolation work, described throughout this Thesis, all isolates were examined under the light microscope to confirm the fact that they were yeasts.

2.11.1. Purified colonies of *Candida* Sp. Isolated from fresh cow dung using CIA medium.

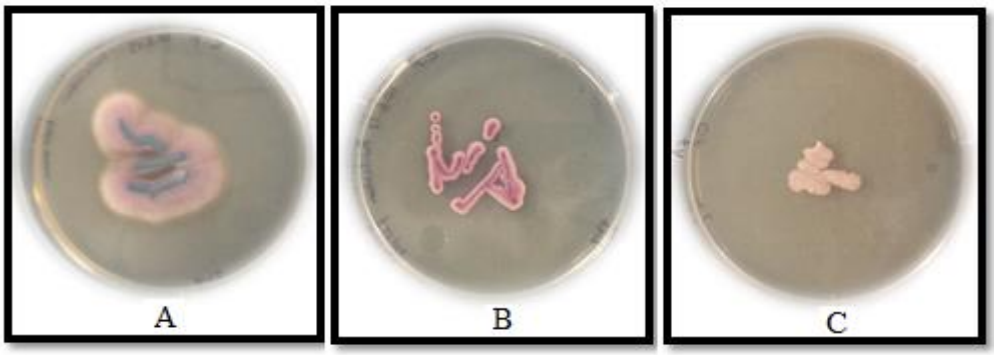


Figure 2.2: CIA plates (A: *Candida xylopsoci*, B: *Candida inconspicua*, C: *Candida rugosa*) showing *Candida* species isolated from cow dung.

Yeasts were isolated from cow dung and genomic DNA of the isolates was successfully extracted using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit). Figure 2.3 A, B shows a Hyperladder 1 was used with success to determine the size of DNA molecules and also shows a success genomic DNA extraction.

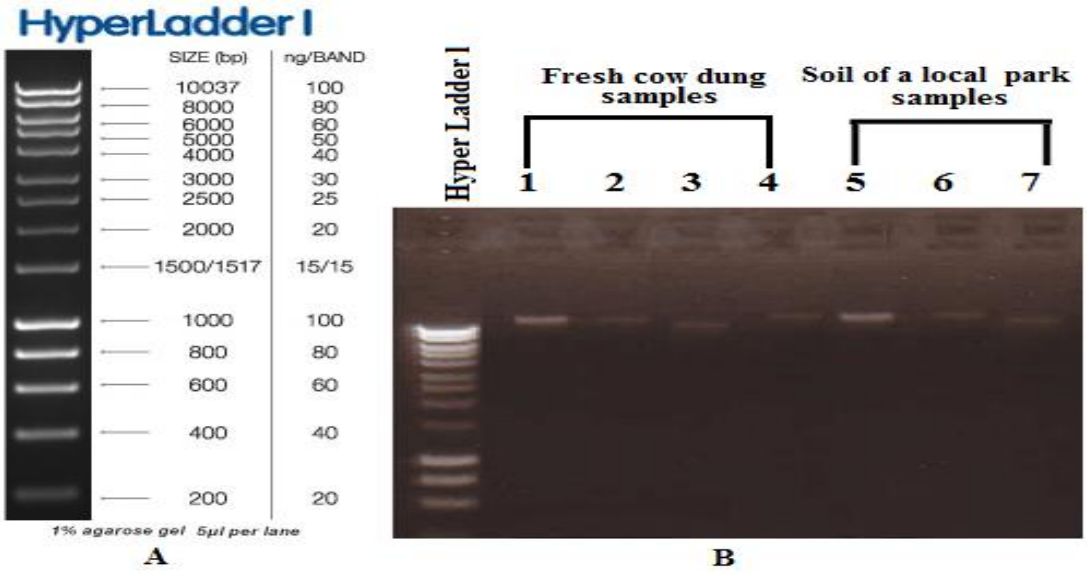


Figure 2.3: Shows the: **A)** Standard Hyperladder I produces of 14 regularly spaced bands and each lane (5 µl) provides 720 ng of DNA. **B)** Successful genomic DNA extraction from unknown microorganisms by the use of 18S rRNA.

2.11.2. PCR amplification of extracted DNA.

Genomic DNA was successfully extracted and the DNA sequence gene of 18S rRNA gene has been determined for seven strains and amplified variable regions. Amplified 18S rRNA genes are shown in (Figure 2.4).

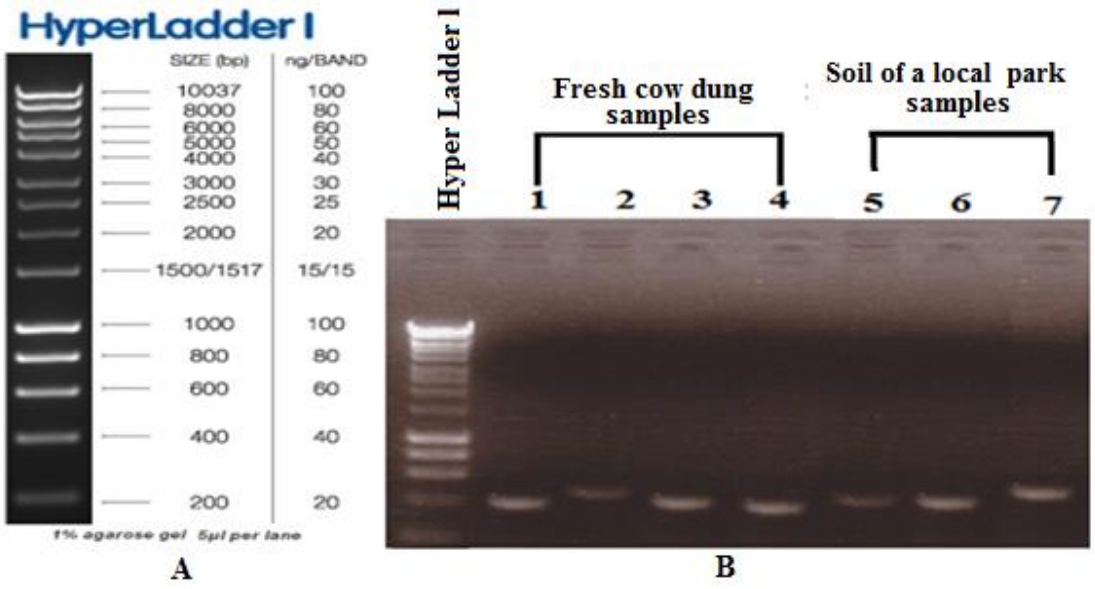


Figure 2.4: Shows: **A)** Standard Hyperladder I produces of 14 regularly spaced bands and each lane (5 µl) provides 720 ng of DNA. **B)** Successful genomic DNA amplification after finishing the DNA extraction processes by using 1% agarose gel electrophoresis for 40 minutes at 85 V.

2.11.3. Phylogenetic identification and analysis of unknown fungi.

18S rRNA sequences resulted from diverse strains were determined after sending them to the University of Sheffield Medical School Core Genetics Unit for further sequencing. BLAST was used to compare the phylogenetic determination of 18S rRNA genes sequences provided from the website of National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) (Altschul *et al.,* 1997). The errors of consensus sequences were corrected manually by using Finch TV software In Finch TV software the unknown nucleotide represents as N, and it could be either A or T or G or C according to the different colours appear (Mishra *et al.,* 2010) (Figure 2.5). The sequences data were used to produce a

phylogenetic tree providing the basis for efficient phylogenetic investigation of each genus) (Figure 2.6). In addition, Table 2.6 shows 18S rRNA analyses representing the closest matches of all yeast isolated.

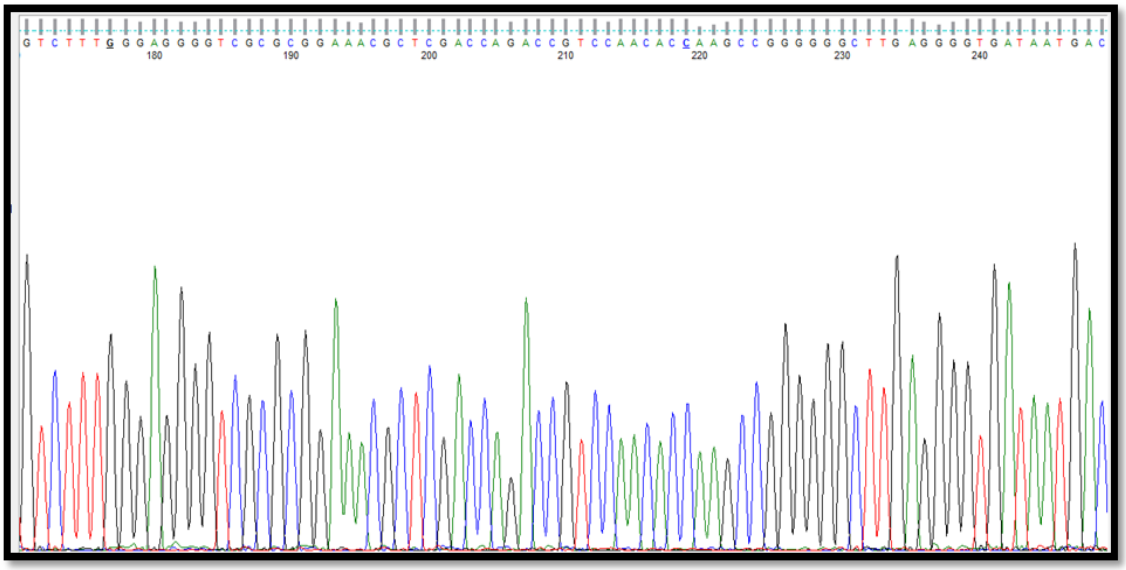


Figure 2.5: Finch TV software (Version 1.4) that allows manual correction of errors, nucleotide represents N and it could be either A or T or G or C according to the different colours appear.

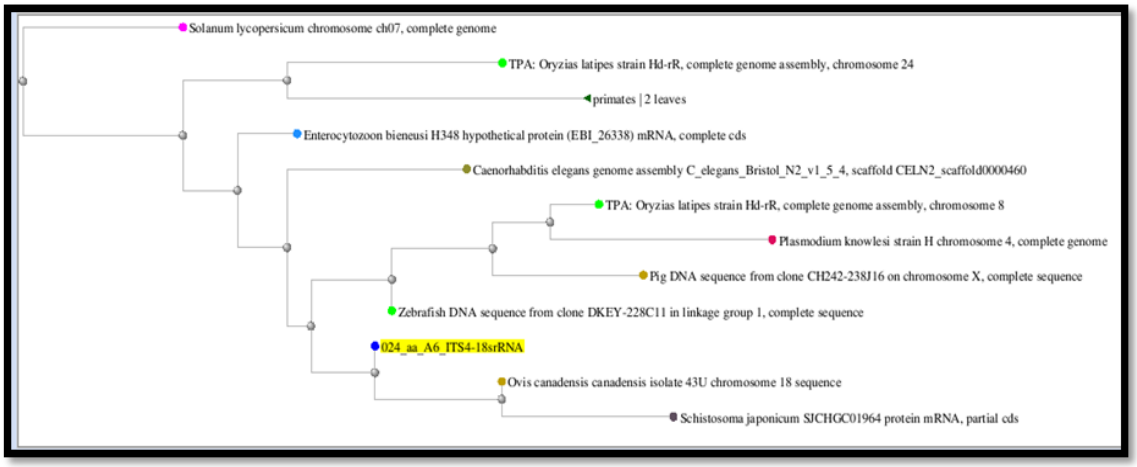


Figure 2.6: Phylogenetic determination of 18S rRNA genes sequences provided from the website of National Centre for Biotechnology Information (NCBI).

Table 2.6: Summary of 18S rRNA sequence analyses of oligotrophic yeast cultured from fresh cow dung and soil.

Samples	Closest matches Identification	Sequence Identity	Sequence ID
Cow dung	*Candida rugosa*	100%	gb|JQ974952.1
Cow dung	*Candida inconspicua*	100%	gb|EU315758.1
Cow dung	*Candida xylopsoci*	60%	emb|FM178339.1
Soil of local park	*Trichoderma asperellum*	50%	gb|KM357296.1
Soil of local park	*Dothideales sp.*	65%	gb|KP963581.1
Soil of local park	*Arthrographis kalrae*	50%	dbj|AB213447.1

2.11.4. Light Microscope images.

After being identified, the yeast isolates were examined under a light microscope (Figures 2.7; 2.8). As was mentioned above, this was necessary because CIA also isolates filamentous fungi.

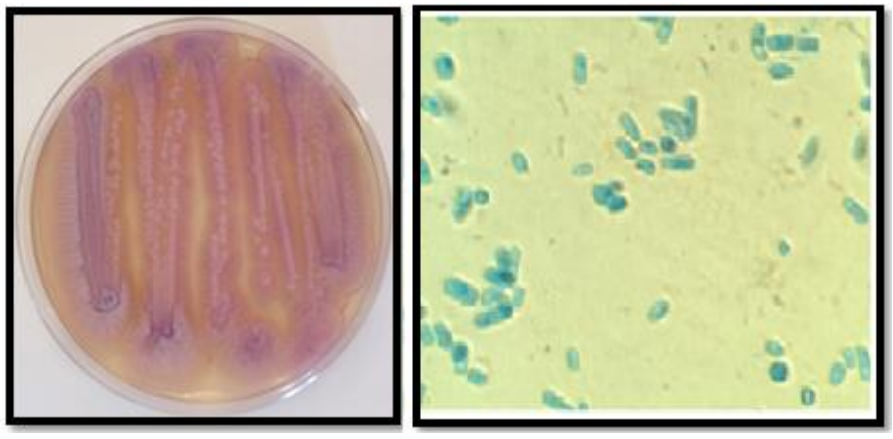


Figure 2.7: Microscope images of ***Candida rugosa*** (stained with lactophenol blue).

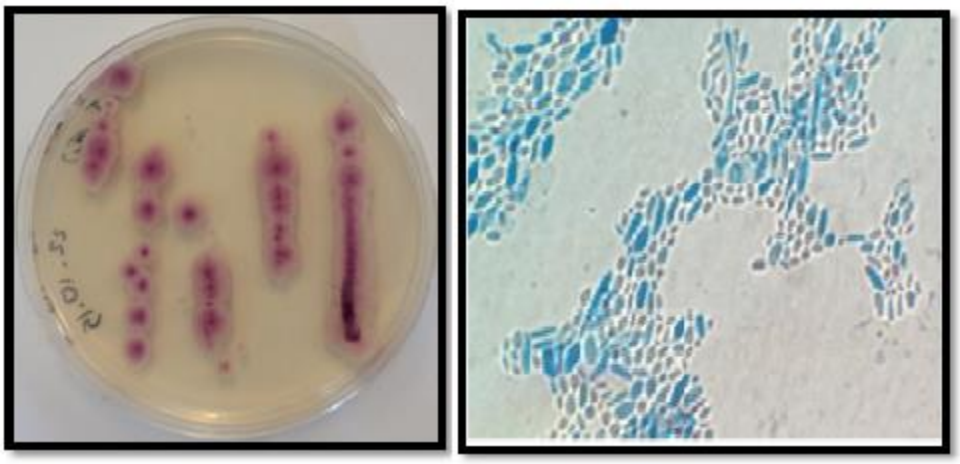


Figure 2.8: Microscope images of ***Candida inconspicua*** (stained with lactophenol blue).

Both yeasts shown in Figures 2.7; 2.8 were identified at the 100% level whereas *Candida xylopsoci* being only isolated at the 60 % level was considered to be a poor match and was not used in further studies.

2.12. Discussion.

Yeast diversity in an environment has traditionally been determined by using various counting techniques which include the plate count technique and other isolation techniques and subsequent identification using classical identification keys. More modern approaches involve the use of 18SrRNA gene sequences (Nannipieri *et al.*, 2003). The molecular analysis of 18S rRNA gene has become dominant in research studies aimed at examining the diversity of eukaryotic microorganisms in the environment (Meyer *et al.*, 2010, Hejazi *et al.*, 2010). Here, genomic DNA was extracted from each yeast isolate obtained from cow dung and then a PCR protocol was used with appropriate primers was conducted to produce sufficient quantities of the 18S rRNA gene. Genomic DNA was isolated by using Norgen's Fungi/Yeast Genomic DNA Isolation Kit (GENEFLOW LIMITED, NORGEN BIOTEK CORP.) by the following procedures suggested for fungi. The genomic DNA was separated by gel electrophoresis on 1% agarose to check for purity. The yeasts, *Candida rugosa*, *Candida*

inconspicua, *Candida xylopsoci* were isolated and identified using 18SrRNA. Since few reports have been published on the environmental occurrence and role of yeast there is little in the way of data which can be used to in comparison with the work presented here. However, both Connell (2005) and Vishniac (2006) found that species of *Crytptococcus*, a yeast not isolated in the work described in this Thesis, was by far the most common soil yeast. Slavikova and Vadertitova (2003) reported isolating some 111 species of yeast from a total of 60 agricultural soils, which begs the question –why were no yeasts isolated from the agricultural soil studied here?

CHAPTER 3

IN VITRO STUDIES ON THE POTENTIAL INVOLVEMENT OF THE YEAST ISOLATES IN COMPONENT PARTS OF THE MAJOR ENVIRONMENTAL MINERAL CYCLES

3.1. Introduction

The aim of the work described in this Chapter was to determine the ability of some of the yeasts (isolated as described in Chapter 2) to participate *in vitro* in some of the important component parts of the major mineral cycles in the environment, namely ammonification, urea hydrolysis, the oxidation of elemental sulphur and the solubilisation of a source of insoluble phosphate. Fungi, including, yeasts (Falih and Wainwright, 1995) have the ability to oxidize elemental sulphur and reduced forms of the element (including metal sulphides Wainwright and Grayston, 1991) *in vitro,* to form oxyanion-intermediates such thiosulphate and tetrathionate (Grayston *et al.,* 1986). In addition it is well-documented that fungi can oxidize reduced forms of nitrogen to nitrate and solubilize insoluble phosphates, although the role of yeasts in these transformations has tended to have been downplayed (Falih and Wainwright, 1995, 2008).

3.2. Materials and Methods.

3.2.1. Standard curve for nitrate determination.

1. 1.37 g of sodium nitrate ($NaNO_3$) was dissolved in 100 ml of distilled water dH_2O to obtain 1µg of nitrate per µl (mixture 1).
2. 10 ml of mixture 1 was diluted into 90 ml of distilled water dH_2O for 10 times (mixture 2).
3. Mixture 2 was diluted with dH_2O to produce solution 0, 10, 20, 40, 60, 80 and 100 µg.

The mixture incubated in water bath for 45 min in 40°C and read with yellow colour at 820 nm by spectrophotometer (Sims and Grant, 1971).

3.2.2. Standard curve for ammonium determination.

1. 3.66 g of ammonium sulphate $(NH_4)2SO_4$ was dissolved in 100 ml distal water dH_2O to obtain 1µg ammonium per µl (mixture 1).
2. 10 ml of mixture 1 into 90 ml of dH_2O to diluted 10 times (mixture 2).
3. Mixture 2 was diluted with dH_2O to produce 0, 10, 20, 40, 60, 80 and 100 µg.

The mixture incubated at 25°C for 20 min in the dark and the blue colour was read at 820 nm using a spectrophotometer (Wainwright and Pugh, 1973).

3.2.3. Standard curve for phosphate determination.

1. 1.48g Na_2HPO_4 was dissolved into 100 ml dH_2O to obtain 1µg of phosphate per µl (mixture 1).
2. 10 ml of mixture 1 into 90 ml of dH_2O to diluted 10 times (mixture 2).
3. Mixture 2 was diluted with dH_2O to produce 0, 10, 20, 40, 60, 80, and 100 µg.

It was incubate at 37°C for 1 hour and blue colour was read at 820 nm using a spectrophotometer (Ajaj, 2005)

3.2.4. Standard curves for sulphate-S determination.

1. 1.47 g $NaSO_4$ was dissolved into 100 ml dH_2O to obtain 1µg of sulphate-S per µl (mixture 1).
2. 10 ml of mixture 1 into 90 ml of dH_2O to diluted 10 times (mixture 2).
3. Mixture 2 was diluted with dH_2O to produce 0, 10, 20, 40, 60, 80, and 100 µg.

It was incubate at 37°C for 1 hour and the white suspension was read at 470 nm using a spectrophotometer (Hesse, 1971)

3.2.5. Determination of *in vitro* urea hydrolysis.

This experiment was performed in order to determine the amount of ammonium released by the yeasts, during urea hydrolysis, when growing in Potato Dextrose Broth liquid medium. The individual yeast isolates were grown in Potato Dextrose Broth liquid medium containing urea (14 g of PDB was dissolved in 600 ml distilled water). The medium was distributed in flasks (50 ml onto 100 ml flasks), and 0.5 g of urea was added directly to each flask medium and sterilized by autoclaving at 120 °C for 20 minutes. On cooling, all flasks, the flasks were inoculated with a one disc from the old culture in triplicate for 28 days at 25°C on a reciprocal shaker (120 revolutions min-1). The pH was measured using a pH meter 3310 at various time intervals (e.g. 0, 7, 14, 21 and 28 days). Ammonium was determined by the indophenol blue method (Wainwright and Pugh, 1973) and nitrate using chromotropic acid (Sims and Jackson, 1971).

3.2.6. Determination of Ammonium

Whatman No.1 filtrate (2mml) was added to 1 ml of EDTA (6% w/v) mixed with 7ml distilled water, 5ml of phenolate reagent and 3 ml of sodium hypochlorite solution (10%v/v). The mixture was then incubated at 25ºC for 20 minutes in the dark after mixing thoroughly. 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. The concentration of ammonium was then finally determined by reference to standard curve prepared from a standard solution of ammonium sulphate.

Reagents:

1) Ethylenediaminetetra-acetic acid (EDTA) ($C_{10}H_{14}N_2O_8NA_2.2H_2O$) :

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

2) Phenol (C_6H_6O) solution: 62.5 g of Phenol was dissolved in 25 ml of ethanol (C_2H_6O) then adding 18.5ml of acetone (C_3H_6O) and made up to 100 ml by distilled water. The phenol solution should store in the dark at 4°C.

3) Phenolate reagent: 20 ml of phenol solution was mixed with 20 ml of hydroxide sodium (25%NaOH w/v) and 60 ml of distilled water to diluting to 100 ml. The reagent was prepared fresh daily.

3.2.7. Oxidation of ammonium to nitrate by yeasts.

This experiment was performed in order to measure the production of nitrate from ammonium by the yeast isolates when grown in Potato Dextrose Broth liquid medium. The medium was distributed in flasks (50 ml onto 100 ml flasks), and 0.5 g of urea ($NH_2CO.NH_2$) was added directly to each flask medium and sterilized by autoclaving at 120 °C for 20 minutes. Then after cooled all flasks, the flasks were inoculated with a one disc from the old culture up triplicate for 28 days at 25°C on a reciprocal shaker (120 revolutions min-1). The concentration of the nitrate was measured at various time intervals (e.g. 7, 14, 21 and 28 days) using the chromotropic acid ($C_{10}H_6O_8S_2NA_2$) method (Sims and Jackson, 1971).

3.2.8. Determination of nitrate.

Whatman No 1 filtrate (3 ml) was added to 7 ml of chromotropic acid (CTA) reagent; the mixture was cooled in cool water then incubated at 40°C in water bath for 45 minutes. The concentration of the nitrate was measured at 630 nm using a spectrophotometer. The concentration of nitrate intensity was then determined by reference to standard curve prepared from a standard solution of nitrate (NaNO3).

Reagents:

1) Stock solution: 1.84 g of chromotropic acid ($C_{10}H_6O_8S_2NA_2$) was dissolved in 1 liter of sulphuric acid (H_2SO_4). The solution was stored at 4°C for several months.

2) Working solution: 100 ml of stock solution in 990 ml of concentrated sulphuric acid (H_2SO_4) then added 10 ml concentrated hydrochloric acid (HCL). The solution was stored at 4°C for several weeks only.

3.2.9. Determination the oxidation of sulphur by yeasts.

This experiment was performed in order to determine the ability of the yeasts to oxidize elemental sulphur. The medium (Potato Dextrose Broth liquid) was distributed in flasks (50 ml onto 100 ml flasks), and 0.5 g of elemental sulphur was added directly to each flask medium and sterilized by autoclaving at 120 °C for 20 minutes. On cooling, the flasks were inoculated with a disc cut a PDA-culture and the flasks were incubated in triplicate for 28 days at 25°C on a reciprocal shaker (120 revolutions min-1). The turbidimetric sulphate method was used to determine the oxidation of sulphur at various time intervals (e.g. 7, 14, 21 and 28 days) (Hesse, 1971). Biomass production in terms of mycelial dry weight and pH of the medium was also determined.

3.2.10. Determination of sulphate.

Whatman No.1 filtrate was transferred to a 50 ml volumetric flask and barium chloride (1g) and (2ml) of gum acacia (0.25% w/v) were added and mixed. The volume was made up 25ml with distilled water. The turbidity was then measured at 470 nm using a spectrophotometer. Sulphate concentration was determined by reference to a standard curve (0-100 μg SO_4 -2-S ml^{-1}) prepared from a standard solution of Na_2SO_4.

3.2.11. Determination of phosphate solubilisation by yeasts.

This experiment was performed in order determine the release, by yeasts, of phosphate from an insoluble phosphate (calcium phosphate $CaPO_4$), when growing in Potato Dextrose Broth liquid medium. The medium was distributed in flasks (50 ml onto 100 ml flasks), and 0.5 g of elemental calcium phosphate $CaPO_4$ was added directly to each flask medium and sterilized by autoclaving at 120 °C for 20 minutes. After cooling, the flasks were inoculated with cultured disc and the flasks were incubated triplicate for 28 days at 25°C on a reciprocal shaker (120 revolutions min-

1). Phosphorus ions were determined at various time intervals (e.g. 7, 14, 21 and 28 days) (Falih, 1995). Biomass production in terms of mycelial dry weight and pH of the medium was also determined.

3.2.12. Determination of phosphate.

Whatman No.1.filtrate (3ml) was added to a 50 ml volumetric flask was mixed with a working solution (7ml) then incubated at 37°C for 1 hour. The concentration of phosphate was measured (blue colour) at 820 nm using a spectrophotometer. Phosphate concentration was determined by reference to a standard curve of (0-100µg PO_4-P ml^{-1}) prepared from a standard solution of Na_2HPO_4.

Reagents:

1. Stock Solution:

A) Ascorbic acid 10g was dissolved in100ml of distilled water.

B) Ammonium molybdate 0.42g was dissolved in 100ml of 1N H_2SO_4 (28ml of H_2SO_4 in 1 litre distilled water).

2. Working Solution: 1 volume of ascorbic acid (10%) was mixed with 6 volumes of ammonium molybdate (0.42%).

3.2.13. Solubilization of insoluble phosphate by yeast growing on solid medium.

Clearing zones of phosphate solubilization produced by yeasts were measured after 6 days of incubation at 28° C on solidified (PGA) Potato Glucose Agar pH 5.6 +/- 0.2 (7.8g PGA + 200 ml distilled water [dH_2O D.W]) medium, supplemented with calcium phosphate (1g calcium phosphate per liter).

3.2.14. Statistics.

All observations are presented as means ± SE (Standard error). The data was analyzed by Sigma Plot© (Version11.0). Paired two or three samples t-test was performed to check whether means were significantly different; $P < 0.05$ was considered as significant.

3.3. Results.

3.3.1. Changes in pH in media amended with urea and inoculated with the individual yeasts.

Figure 3.1 show that the medium pH at week 1 was as follows: *Candida rugosa* pH 9.3, *Candida inconspicua* pH 9.3 and the control pH 9.0. The pH decreased rapidly in medium in which *Candida rugosa* was growing at week 2, 3 and 4 (pH 8.9, pH 8.7 pH 7.8). At week 2, 3, 4 there was little difference between *Candida inconspicua* and control. The pH values over the incubation period were as follows: *Candida inconspicua* pH 9.1, pH 8.7, pH 8.3 and control pH 8.9, pH 8.3, pH 8.0; the pH of the medium in which *Candida rugosa* was growing and the control decreased gradually after week 3.

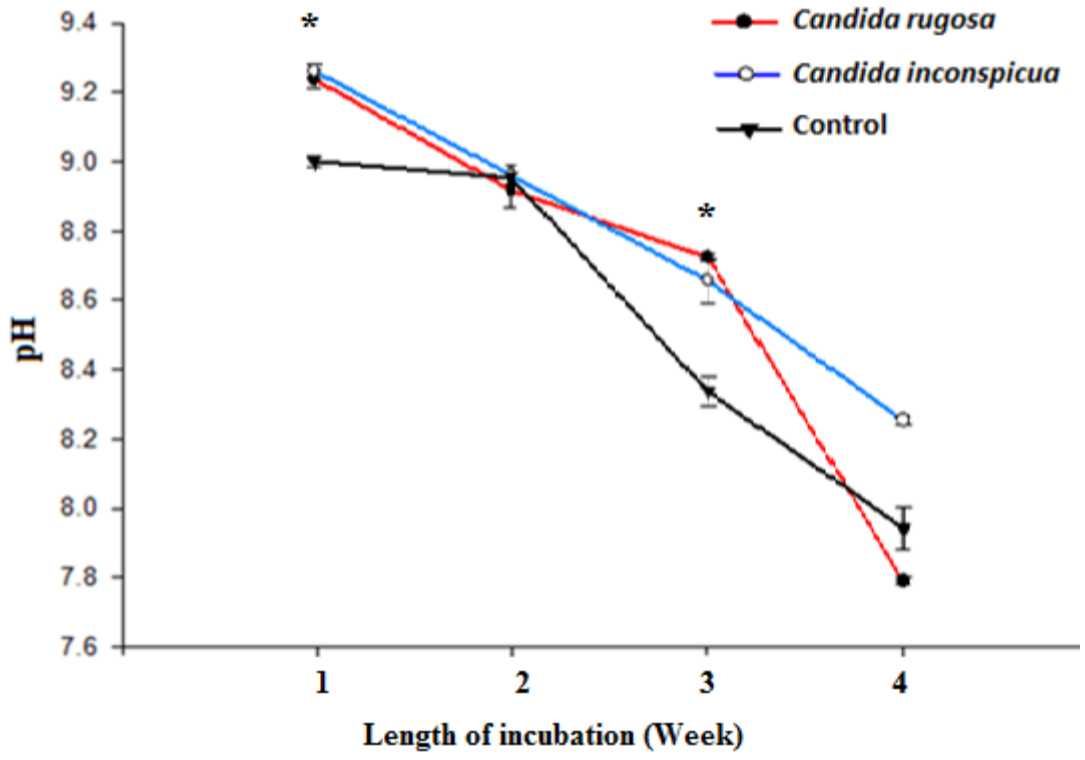


Figure 3.1: pH during urea hydrolysis *Candida rugosa,* *Candida inconspicua* and Control (without yeast).

Standard error (SE): ***** significant difference from control P<0.050.

3.3.2. Determination of the urea hydrolysis to ammonium by yeasts.

Figure 3.2 shows that both yeasts were capable of hyrdolysing urea over the incubation period.

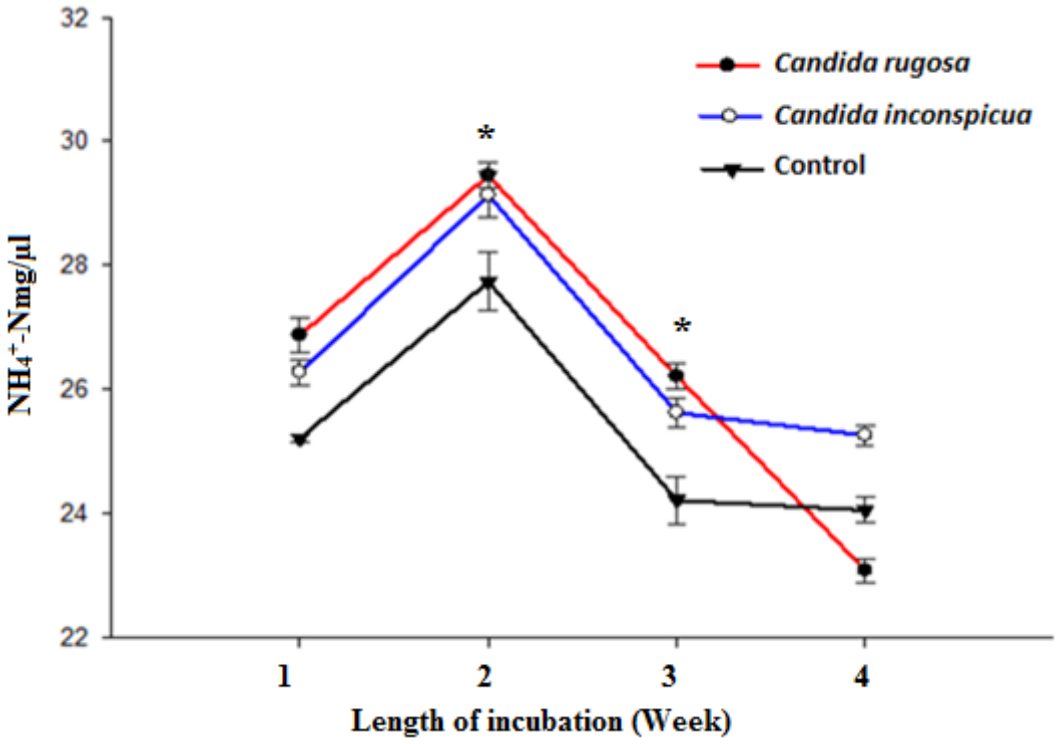


Figure 3.2: Urea hydrolysis to ammonium by the yeasts *Candida rugosa*, *Candida inconspicua* and Control (without yeast).

Standard error (SE): ***** significant difference from control P<0.050.

3.3.3. Changes in pH in media amended with urea and inoculated with the individual yeasts.

Figure 3.3 shows that there was no difference in pH between the yeasts over the length of the incubation period. Values of pH at week 1 were all the same, while the pH decreased rapidly in medium in which *Candida rugosa* was growing at week 2, 3 and 4 as follows: pH 8.1, pH 7.3 and pH 5.8. At week 2, 3, 4 it there was no difference in pH in media supporting *Candida inconspicua* and the control. The pH values were as follows: *Candida inconspicua* pH 9.2, pH 8.6, pH 7.9 and control pH 8.9, pH 8.7, pH 8.1.

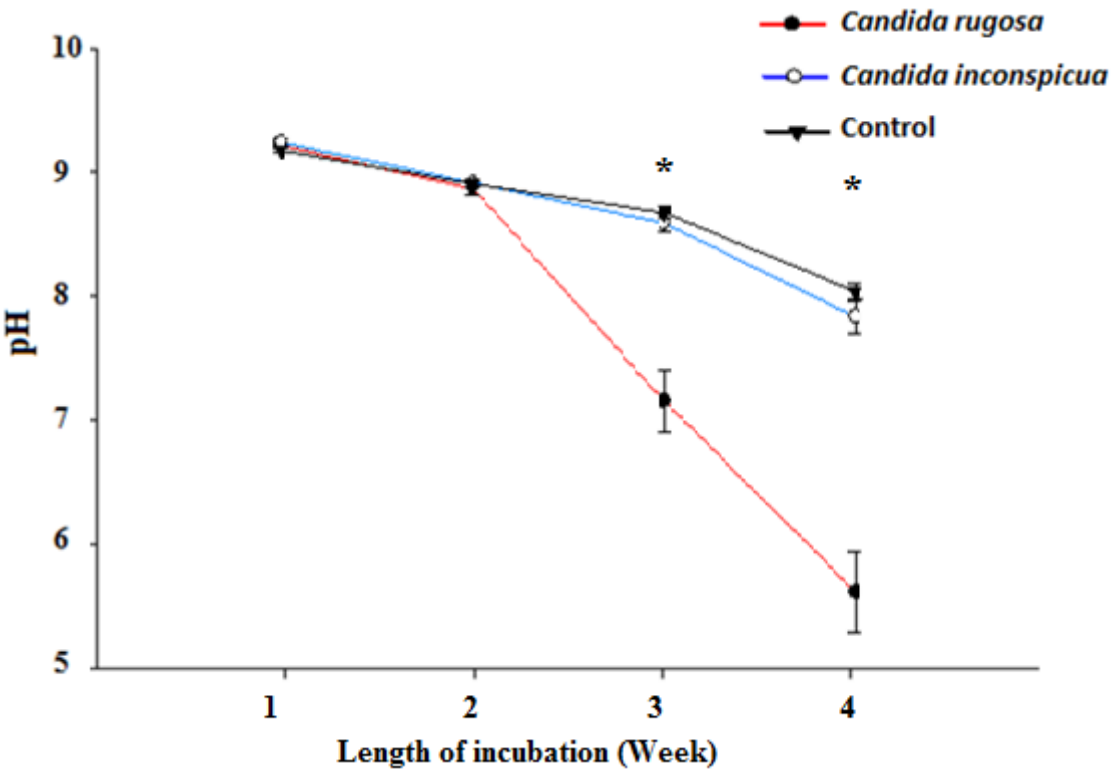


Figure 3.3: pH of medium in containing urea *Candida rugosa*, *Candida inconspicua* and Control (without yeast).

Standard error (SE): ***** significant difference from control P<0.050.

3.3.4. Determination of the oxidation of ammonium to nitrate by yeasts.

Figure 3.4 shows a comparison the oxidation of ammonium to nitrate between the two species of yeast isolated from cow dung and the control over the four week incubation period. It can be clearly seen that both yeasts oxidized ammonium to nitrate, particularly at week 2 in the case of *C. rugosa*; this yeast tended to be more active in the process than was *C. inconspicua.*

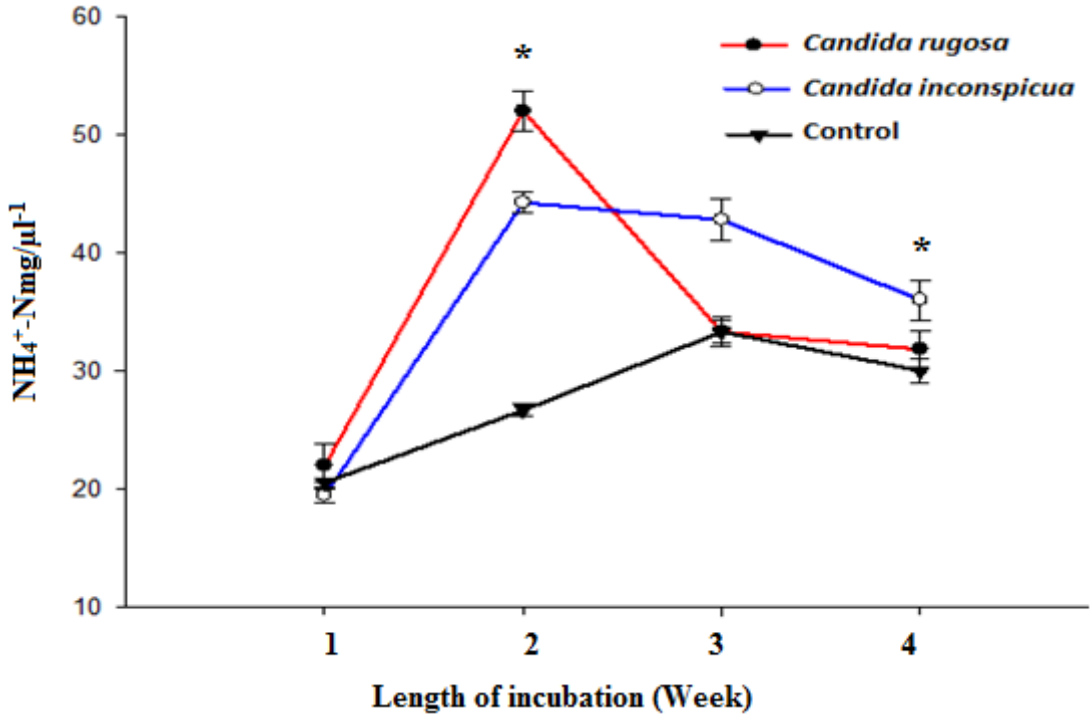


Figure 3.4: Oxidation of ammonium to nitrate by yeast *Candida rugosa*, *Candida inconspicua* and Control (without yeast).

Standard error (SE): ***** significant difference from control P<0.050.

3.3.5. Changes in pH in media amended with elemental sulphur and inoculated with the individual yeasts.

Figure 3.5 shows pH values during sulphur oxidation by the two yeasts and the control over the four week incubation period. It can be seen the pH there was difference between the yeast and the control over the length of the incubation period. The pH at week 1 was as follows: *Candida rugosa* pH 4.0, *Candida inconspicua* pH 4.0 and the control pH 3.9. While the pH for *Candida rugosa* decreased gradually at week 2 and increased at week 3, 4 as follows: pH 3.9, pH 4.5 and pH 4.7. At week 2, 3, it shows clear increases in the pH for *Candida inconspicua* as follows: pH 4.1, pH 4.6 and at week 4 there was decreased slightly in the pH 4.5. Whilst control values increased steadily over the week 2, 3, 4 as follows: pH 3.9, pH 4.3, pH 4.6; medium pH for *Candida rugosa* and controls increased at weeks 2, 3, 4.

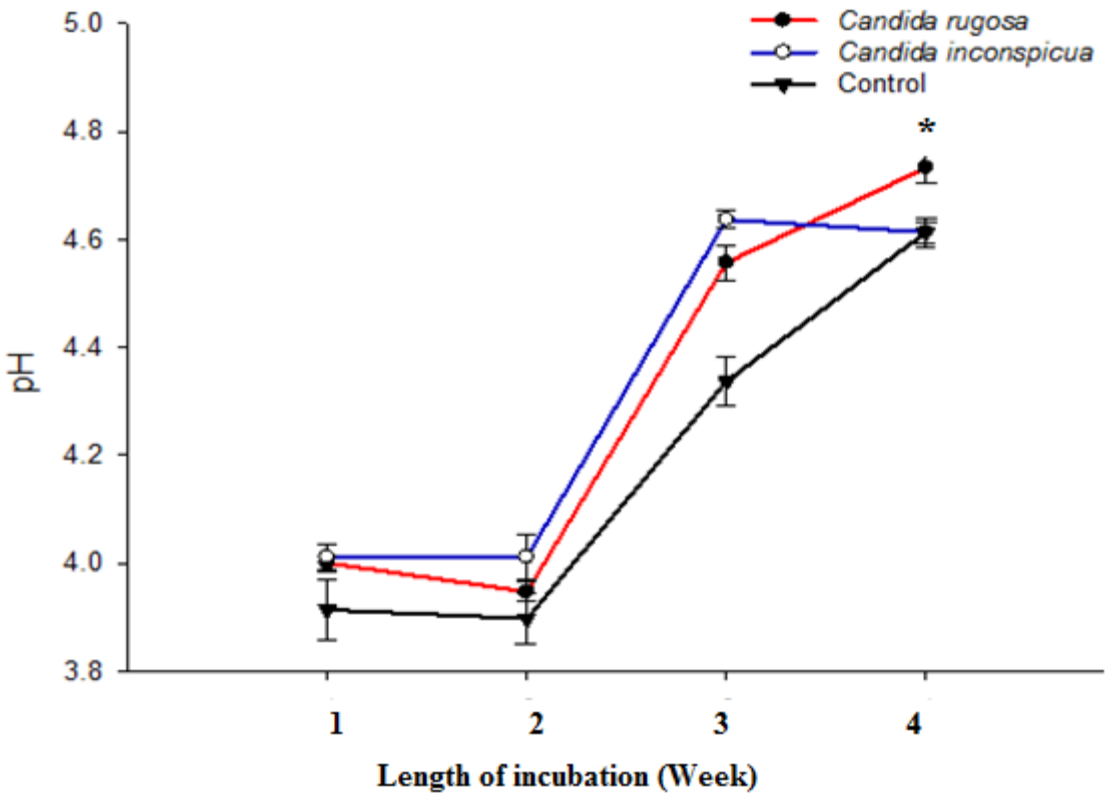


Figure 3.5: pH during S-oxidation *Candida rugosa* *Candida inconspicua* and Control (without yeasts).

Standard error (SE): ***** significant difference from control P<0.050.

3.3.6. Determination the oxidation of sulphur by yeasts.

Microbial sulphur oxidation to sulphate occurred in media in which grew the two types of yeast isolated from cow dung. Figure 3.6 shows the two yeasts oxidized sulphur; *Candida inconspicua* being the most active of the two yeasts in this respect.

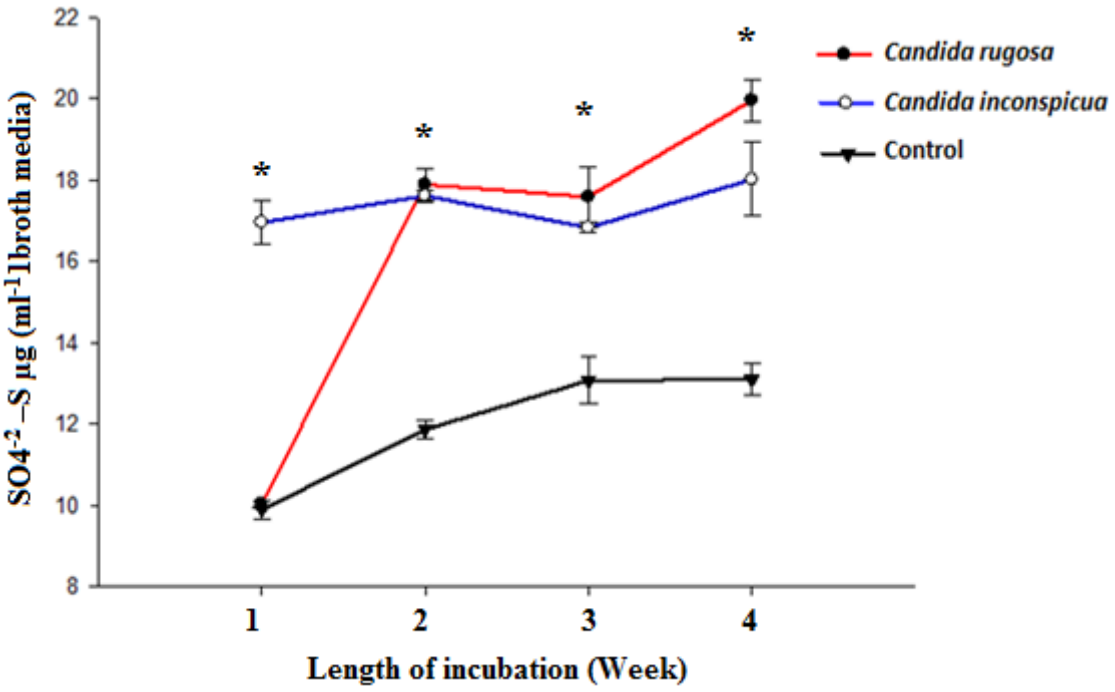


Figure 3.6: Oxidation of sulphur by yeasts. *Candida rugosa* *Candida inconspicua* and Control (without fungi).

Standard error (SE): ***** significant difference from control P<0.050.

3.3.7. Changes in pH in media amended with insoluble phosphate and inoculated with the individual yeasts.

Figure 3.7 shows medium pH during phosphate solubilisation in the medium for the two yeasts isolated from cow dung and the control over the four week incubation period. The pH of medium in which the yeasts were growing was higher than the control value over the entire incubation period.

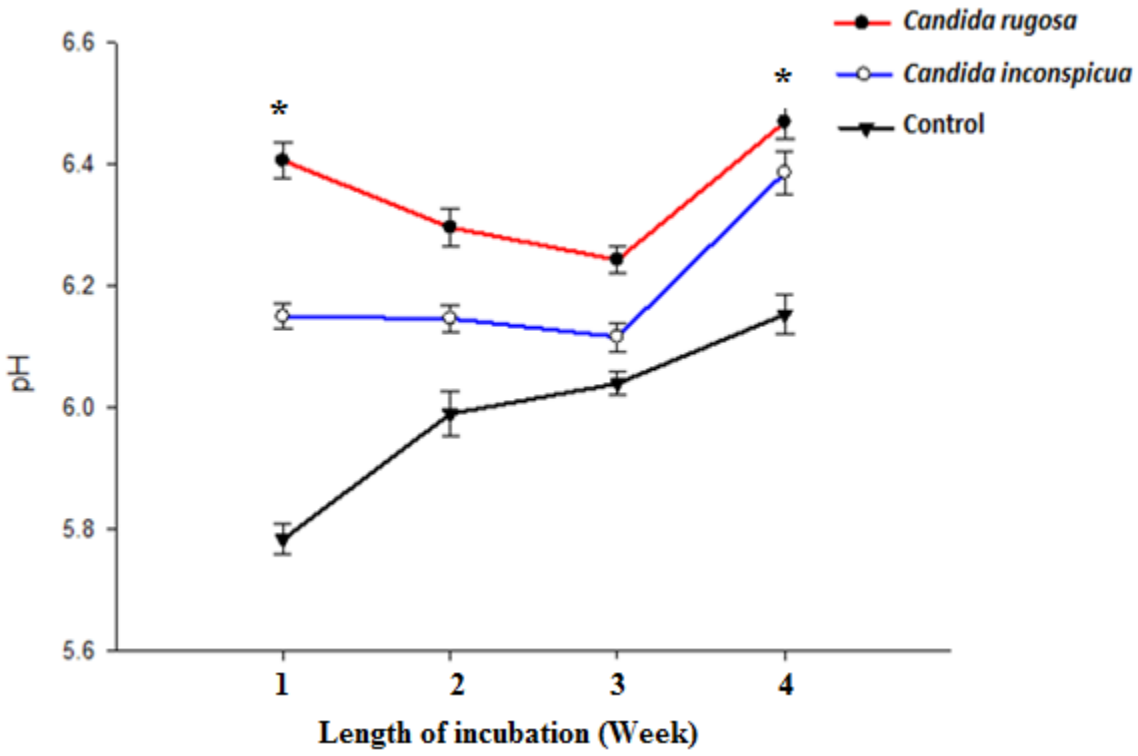


Figure 3.7: pH during P-solubilisation *Candida rugosa* *Candida inconspicua* and Control (without yeasts).

Standard error (SE): ***** significant difference from control P<0.050.

3.3.8. Solubilisation of insoluble phosphate by the two yeasts.

Figure 3.8 shows phosphate solubilisation by the yeasts over the four week incubation period. The results show that the two yeast were both able to solubilize insoluble phosphate in liquid medium

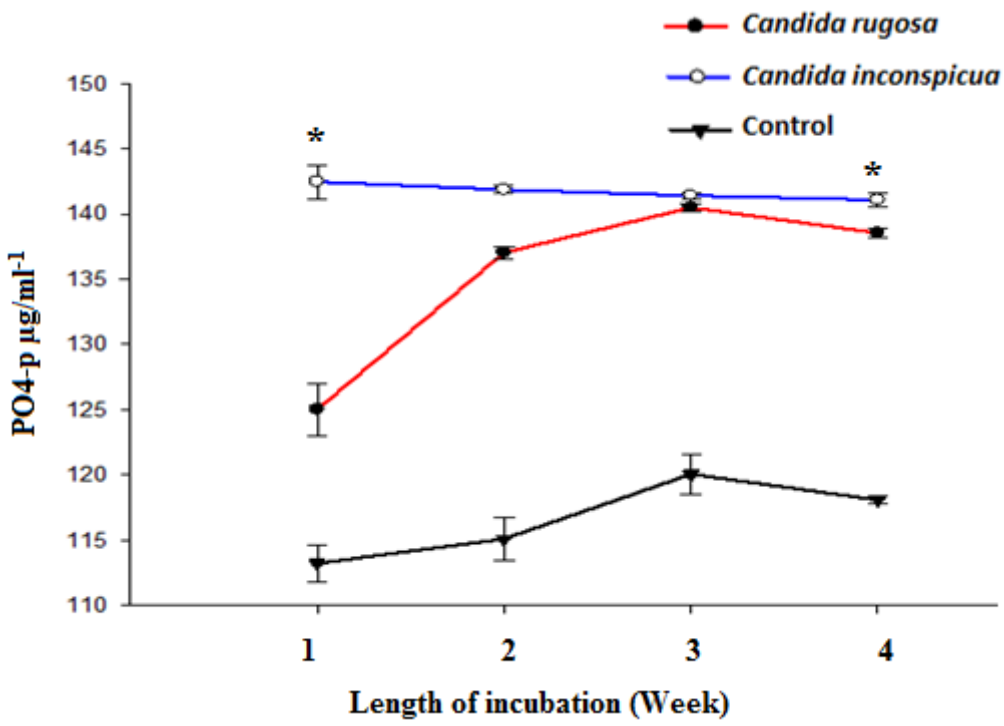


Figure 3.8: Solubilisation of phosphate by yeasts. *Candida rugosa,* *Candida inconspicua* and Control (without fungi).

Standard error (SE): ***** significant difference from control P<0.050.

3.3.9. Yeast solubilisation of insoluble phosphate on solid medium.

The yeast *C. rugosa* did not produce a noticeable zone of clearing after 6 days of incubation at 28° C, indicating lack of phosphate solubilizing ability in this yeast. A clear zone was however formed around the colonies of *Candida inconspicua* (Figure 3.9)

Figure 3.9: Yeast-solubilisation of inorganic phosphorus. **A-** a clear zone was formed around colonies of *Candida inconspicua*. **B-** absence of clear zones were formed around colonies of *Candida rugosa.*

3.4. Discussion.

The results presented in this Chapter show that yeasts can be isolated from bovine dung, but not the single soil sample tested. The fact that the yeasts were isolated using Candida isolation medium shows that it is effective for isolating these organisms from the environment. Little more can be said however, about the absence of yeasts in the soil tested in relation to the general presence of yeasts in soils, simply because not enough soils were tested. However, the fact that no yeasts were isolated from the inorganic agricultural soils, but were obtained from the cow dung, fits in with the general view that yeasts prefer rich organic rich environments, or ones which have been naturally amended with sugars, e.g. leaf or aphid exudates, or as the result of agricultural management, e.g. soils receiving sugar beet toppings or sugar beet amendment (Wainwright and Falih, 1995). The results of the present study show that the two yeasts isolated from the bovine dung were able to mediate important component transformations of the major mineral cycles, namely, urea hydrolysis, and the oxidation of

ammonium and sulphur and the solubilization of a source of insoluble phosphate. Similar findings have been reported in the literature for soil fungi and yeasts, including *Williopsis californica* (Falih and Wainwright, 1995, 1995b). However, these results reflect media-based studies which, it could be argued, tell us little about the ability of microorganisms to participate in biogeochemical processes in the environment. This is essentially because of the use here of a nutrient–rich medium, i.e. Potato Dextrose Broth (PDB). Nearly all similar investigations of the role of heterotrophs, such as filamentous fungi and yeasts involve the use of defined media such as Czapek Dox medium. However, in the present described studies a semi-defined medium, PDB was used. This choice was based on the fact that the yeast isolates failed to grow on defined media, but grew well on PDB which, by containing potatoes (and dextrose) is marginally closer to a naturally occurring carbon substrate than is the 30 g per liter of sucrose found in Czapek Dox medium. The biogeochemical processes studied here are traditionally associated with chemolithotrophic bacteria and the potential role of heterotrophs in their mediation has generally been played down at least in textbooks and non-specialized environmental texts. However, over the last thirty or so years the potential role of bacteria and filamentous fungi in these processes has come under increasing scrutiny. It is now generally accepted that heterotrophs play an important role in processes such as nitrification and sulphur oxidation in acidic and highly organic soils, such as coniferous forests, where the role of chemoautotrophs will be relatively less important (Grayston *et al.* 1986, Grayston and Wainwright, 1986). However, in the case of nitrification, this view may be based on the false assumption that nitrifying bacteria cannot function in acidic soils. This view has however, been challenged following the recognition that acid tolerant *Nitrosomonas* and *Nitrobacter* exist. It may well be however, that the importance of heterotrophic nitrification and S-oxidation to the organisms involved is not directly related to the formation of end products which are so important (because of their fertilizer value to agriculture), but to the indirect benefits bestowed

on a heterotroph in participating in these processes. For example, the production of polythionates, by fungi, during S-oxidation may help protect these organism from the toxic effects of heavy metals and other toxicants.

Recently, considerable research effort has been devoted to the study of insoluble phosphate solubilization by filamentous fungi and bacteria. The result presented in this Chapter, confirm that yeasts can solubilize insoluble phosphate. It is interesting to not however, that *C. rugosa* ,while it solubilized insoluble phosphate in liquid medium, did not do so when growing on solid medium amended with phosphate. The reason for this is not clear, but may be related to the question of contact between the phosphate particles and the yeasts, this being more effectively achieved in a shaking medium. The increased research effort is based on the fact that phosphate is likely to be increasingly, and worryingly, limiting in most of the world's agricultural soils within the near future. This has lead to the study of the possible use of microbial inoculants to enhance the process, most notably in the rhizosphere. As has already been mentioned the likely preference exhibited by yeasts for carbon-rich environments is likely to restrict the use of these organism for this purpose. This potential problem might be overcome by the addition, to inoculants of a cheap carbon–rich substrate such as sugar beet bagasse. The increasing recognition of the potential importance of phosphate solubilizing microorganisms has lead to a recent return of interest in these organisms, as is evidence by the developing literature on there involvement in this process. Because of the increasing importance of phosphorus availability in agricultural soils, a relatively detailed account of the role of microorganisms in P-solubilisation follows:

Numerous heterotrophs solubilize insoluble phosphate from a variety of sources. Phosphate is assimilated by these microbes and coincidently solubilized making P available to other organisms, notably crop plants. Various chemolithotrophic microbes e.g. *Nitrosomonas* and *Thiobacillus* also mobilise inorganic P by the production of nitric and

sulphuric acids, respectively (Tiessen and Stewart, 1985). A number of reports have detailed the ability of bacteria to solubilize insoluble inorganic phosphate compounds, notably tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate (Altomare *et al*, 1999; Baijpai *et al*., 1971; Goldstein,1986; Nannipieri *et al*. 2011; Venkateswarlu *et al*. 1984; Wu *et al.* 2009; Anamika *et al.* 2011), including species of *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium* and *Erwinia* (Hilda and Frega, 1999). Parks, *et al*. (1990) showed that microbes can solubilize insoluble phosphate as a means of removing phosphate contaminants from iron ore, an ability attributed to the production of organic acids (Kucey *et al*., 1987; Molla, *et al*., 1984; Nahas, *et al*, 1990; Agnihotri, *et al*.1970; Kpomblekou and Tabatabai 1994). The mechanisms of solubilisation of insoluble phosphate are related mostly to the acidification of the medium and organic acid production, notably glycolate (Banik and Dey, 1982; Goldstein, 1986; Cunningham and Kuiack, 1992; Goldstein, 1995; Gyaneshwar *et al*., 1998; Kim *et al*., 1997, 1998; Deubel *et al*., 2000). Fungi also produce phosphatases in order to mineralise organic-P (Pandey, *et al*., 2008; Vassileva, *et al*., 2010). In conclusion, it is generally accepted that mineral phosphate solubilisation results from the synthesis of organic acids by soil microorganisms which results in the acidification of media **(**Chen. 2006; Uroz *et al*, 2007) for examples showed that *Aspergillus niger* produces gluconic acid, oxalic acid; while *Penicillium* Spp. produce malic acid, gluconic acid and oxalic acids (Helmut B. and Mohammad A. F., 2006). Malial (2004) also showed that *A. niger*, *A. flavus* and *P. canescens* produce citric, and other workers have added to this list gluconic, oxalic and succinic acids (Puente *et al*. 2004, Rodriguez *et al*. 2006). Studies on phosphate solubilisation involve isolating microorganisms from the soil and other environments and then studying their solubilisation ability *in vitro*. Phosphate–solubilizing bacteria have been used as an agricultural biofertiliser; in the former Soviet Union for example, a commercial

biofertiliser under the name “phosphobacterin” was prepared using *Bacillus megaterium* var. *phophaticum*; it is was widely used in Soviet East European countries and India (Smith *et al*., 1962). Phosphate solubilizing microorganisms (fungi and bacteria) include species of *Pseudomonas, Mycobacterium, Micrococcus, Bacillus, Flavobacterium, Penicillium, Sclerotium, Fusarium, Aspergillus* (Alexander, 1977). Up to 85% of the microbes in some soils can solubilize phosphates, although the ability is often lost on sub-culturing. The rhizosphere often exhibits a particularly high proportion of such organisms; Swaby and Sperber (1959), for example found that 20 – 40% of the bacteria, actinomycetes and fungi isolated from the rhizospheres of many plants can to dissolve hydroxyapatite, compared to 10–15% of those isolated from non–rhizosphere soil. Katznelson *et.al.*, (1962) isolated a species of *Candida* from soil which solubilized insoluble phosphate. P-solubilizing filamentous fungi include species of *Aspergillus, Fusarium, Penicillium* and *Sclerotium* (Alexander, 1977) which solubilise calcium phosphate, apatite or similar insoluble phosphates and release soluble P into the medium (Al-Turk, 1990) as do most species of bacteria, including actinomycetes. Certain species of bacteria and fungi can also produce hydrogen sulphide, which reacts with ferric phosphate to produce ferrous sulphide and soluble phosphate (Hattori, 1973). Inorganic P compounds are also solubilised by the reaction of organic and inorganic acids which are secreted by fungi and bacteria in hydroxyl and carboxyl groups of acids from which chelate cations (Ca, Al and Fe) and reduce soil pH (Kpomblekou and Tabatabai, 1994; Banik and Dey, 1982; Goldstein 1986; Cunningham and Kuiack, 1992; Goldstein, 1995; Gyaneshwar *et al*., 1998; Kim *et al*., 1997, 1998; Deubel *et al*., 2000). Phosphate solubilisers can be detected by their ability to produce clearing zones around colonies in media which have been amended with insoluble mineral phosphates (notably calcium phosphate or hydroxyapatite) as the sole P source, an approach which can be visually improved upon by adding bromophenol blue to the medium as a pH indicator;

acidification of the medium by phosphate solubilizers forms, yellow-coloured halo around the colonies in response to the decline in pH and the release of organic acids resulting from phosphate solubilisation. *Rhizobium, Pseudomonas* and *Bacillus* species are among the most active solubilizers of inorganic P, while tricalcium phosphate and hydroxyapatite are more readily degraded than is rock phosphate (Sharma *et al.*, 1995).

CHAPTER 4

THE DISTRIBUTION OF *CANDIDA* AND YEASTS IN THE ENVIRONMENT AND THEIR SURVIVAL IN HEALTHCARE SETTINGS.

4.1. Introduction.

As we have seen, relatively little is known about the role of yeasts in the environment and the factors which influence their survival. This relative lack of information is potentially damaging since many yeasts (notably species of *Candida*) act as animal and human pathogens, especially in immunocompromised patients. Little is known about the survival of fungi causing nosocomial infections in compromised patients on typical hospital materials (Blaschke-Hellmessen, *et al.,* 1989; Rangel-Frausto *et al.,* 1994). Many fungi (*Candida, Aspergillus, Mucor*, and *Fusarium*) which are associated with nosocomial infections in patients can survive for at least a day and often longer on fabrics and plastics routinely used in hospitals Blaschke-Hellmessen *et al.,* (1989), the length of survival of the fungi depending upon both the genus and species tested and on the specific surface upon which the fungi were inoculated.. Blaschke-Hellmessen *et al.,* (1989), also found that common environmental yeasts, such as *Rhodotorula* spp., are more resistant to drying than yeasts associated with mucous membranes, e.g. *C. albicans*. These results show the potential for various fabrics and plastics to act as reservoirs or vectors for yeasts, with the species tested generally remaining viable on these surfaces for at least a day and often for weeks. Rangel-Frausto *et al.* (Rangol-Frausto *et al.,* 1994), showed that when dried onto plasic, *C. albicans* could be transferred to the hands of test volunteers. Fungi can therefore exist for periods on common hospital fabrics and plastics, a fact which is of some considerable concern in an age of increasing antibiotic resistance; conscientious contact-control procedures are therefore clearly essential for the successful control of infections in hospitals (Rangol- Frausto *et al.,* 1994).

A major aim of the work described in this Thesis is to determine the factors which influence the survival of *Candida* in the built environment (e.g. on ceramic tiles, in washrooms and in toilets) notably in relation to the survival of pathogenic yeast in hospitals and other medical situations. Nosocomial bloodstream infections present a serious medical problem and are associated with significant mortality and health care costs. These diseases are primarily caused by *Candida* species, are now considered to be the fourth most common blood stream infections in the US (Morell *et al.,* 2005). Risk factors for the development of *Candida* bloodstream infection and include the previous administration of antimicrobial agents, corticosteroids, or a variety of chemotherapeutic agents; hematologic or solid-organ cancers; neutropenia; extensive intra-abdominal surgery or burns; mechanical ventilation or admission to an intensive care unit; indwelling central venous catheter or parenteral nutrition; hemodialysis; and finally, prior yeast colonization. There has recently also been an increase in the number of non-*Candida albicans* species associated with bloodstream infection. Earlier exposure of the patient to anti-yeast therapy, notably with the antibiotic fluconazole, seems to be an accurate predictor for bloodstream infection with non-*C.albicans*-*Candida* species. Appropriate initial antimicrobial therapy is recognized as an important predictor of outcome for patients with microbiologically confirmed nosocomial infections, including bloodstream infections and severe sepsis. The incidence of systemic candidosis is increasing, a trend which is likely to continue with increases in the population of susceptible patients. Mortality is high (75%) despite treatment with amphotericin B, which is unfortunately nephrotoxic. Chemoprophylaxis with oral anti-yeast agents has long been used in patients at high risk of infection, as the gastrointestinal tract is regarded as the major source of invasive candidosis (Burnie *et al.,* 1985). High dosages of oral nystatin, amphotericin B, or ketoconazole reduce Candidal colonisation and may decrease the incidence of invasive candidosis in neutropenic patients. Unfortunately, no type of chemoprophylaxis is completely satisfactory. Invasive

candidosis is usually attributed to autoinfection by yeasts colonising the patient's bowel or intravenous catheters (Burnie *et al.,* 1985).

In the US, the incidence of bloodstream infections with *Candida* species is 6 to 14 per 100,000 persons per year and the rate of mortality associated with this yeast can approach 50%. Almost 80% of these infections, including those in the intensive care unit and outpatient settings are found in persons receiving a central venous catheter (CVC). Outbreaks and clusters of cross-transmission, both in hospital and in out-patients, appear to be due to extrinsic contamination, intravascular devices, and various medications. Although the environment is a well-known source of human infection by *Aspergillus* species (Raud 2004; Anaissie and Costa, 2001; Carter and Barr, 1997; Leenders *et al.,* 1999), few data exist on the environmental sources of human pathogenic yeasts. The important major opportunistic infections are caused by *Cryptococcus spp., Histoplasma capsulatum and Rhodotorula* spp., yeasts which are widespread in the environment and, like *Candida,* have been isolated from animals (notably birds and mammals) and also from sea water and trees (Blaschke-Hellmessen, 1999, 2000; Camin *et al.,* 1998; De Vroey, 1979; Odds, 1984; Younglove *et al.,* 1968). The survival of yeasts outside the host is poorly understood (Koike *et al*., 1992; Odds, 1991; Valdes-Collazo *et al*., 1987). The authors found that both *Cryptococcus* Spp. and *Candida albicans* survive well in the environment, i.e. more than 24 weeks in mix of soil and water, at 20^{o}C and 30^{o}C (Theraud *et al*., 2003). Yeast cells present in biofilms are markedly more resistant to anti-yeast agents used in human therapy (Chandra *et al.,* 2001b; Hawser and Douglas, 1995; Ramage *et al.,* 2001) than are planktonic yeast cells. Tests against biofilms by the above named, showed that eight out of nine biocides were ineffective, with chlorhexidine at 0.5% being the only fungicide which was effective against pure cultures, yeast mixtures as well as biofilms. A significant proportion of hospital infections are caused by cross-contamination and transmission of microbes from; hospital surfaces (Cesar-Pastuor *et al.,*

2012) the hands of health care workers and various medical equipment which has become contaminated with a range of both pathogenic and non-athogenic organisms. Common human pathogenic bacteria, notably *Escherichia coli*, *Enterococcus* spp, *Acinetobacter* spp, *Staphylococcus aureus* and noroviruses can survive for long periods on hospital surfaces or fomites from where they can potentially infect patients. Additionally, hospital infections can be caused by fungi, including *Candida* spp and species of *Aspergillus*, *Cladosporium* and *Penicillium* (Thomas *et al.,* 2004; Peter *et al.,* 2003). Air ventilator systems (HEPA filter and common filter) also act as source of fungi such as *Penicillium*, *Aspergillus*, *Cladosporium*, *Trichoderma*, *Stereptomyses*, *Chrysosporium* and *Rhizopus*. Therefore it is important to know about the location of these potential pathogens in different areas of a hospital and also to implement monitoring regimes so as to determine the effectiveness of aseptic approaches and thereby better prevent infectious diseases caused by nosocomial pathogens.

4.2. Isolation of yeasts from sinks, toilets and hospital and natural environments.

The hospital environment is potential reservoir of pathogens (Dancer, 2009). Nosocomial infections are acquired during hospitalization and often result from the transmission of microorganisms (bacteria and fungi) from; surfaces, sinks, hands of health care workers and medical equipment (Bauer *et al.,* 1990, Kayabas *et al.,* 2008, Medina *et al.,* 1997, Sehulster *et al.,* 2003). Hospital infections can also be transmitted by ventilator systems (e.g using HEPA filters) from which filamentous fungi such as *Trichoderma*, *Penicillium*, *Cladosporium*, *Aspergillus* and *Chrysosporium* have been isolated (Faure *et al.,* 2005, Lajonchere, 1994). Hashemi *et al.,* 2004; Sehulster *et al.,* 2003 reported that *Candida spp*. are common nosocomial infective organism in many areas of the world (see also, Pfaller, 2007, Pfaller *et al.,* 1998). During the 1980s, (Pfaller) the frequency of nosocomial candidiasis increased dramatically. This trend has continued into the 1990s, and *Candida* species remain a major cause of nosocomial infections. Although *Candida albicans* remains the most frequent cause

of fungemia and hematogenously disseminated candidiasis, a number of reports have documented infections caused by other *Candida* species, including: C. *tropicalis,* C. *glabrata,* C. *parapsilosis,* C. *krusei,* and C. *lusitaniae.* Many of these infections arise from an endogenous source, and their frequency is influenced by the patient population, the various treatment regimens, and the antibiotics or other supportive care measures employed at specific institutions. Additional infections may be accounted for by exogenous acquisition via hands of health care workers, contaminated infusates and biomaterials, and the inanimate environment. Ongoing investigations should help improve our understanding of the epidemiology of candidiasis and allow for the development of rational preventive measures.

Candida species are pervasive pathogens capable of causing both local and systemic infections in hospitalized patients (Seneviratne *et al.,* 2008). These organisms are now well-established as important nosocomial pathogens in seriously ill immunocompetent patients (Burnie *et al.,* 1985; Thomas *et al.,* 2004, Matthew *et al.,* 2005; Thomas *et al.,* 2004; Seneviratne *et al.,* 2008). The literature shows that *Candida* species are the fourth most common nosocomial pathogens in intensive care units and accounted for 10% of all bloodstream infections and 25% of all urinary tract infections in the intensive care unit setting (Comert *et.al.,* 2007). The frequency of nosocomial candidemia has increased dramatically over the decade from 1980 through 1989. David *et al.,* (2010) reviewed trends in nosocomial bloodstream infections among hospitals surveyed as part of the National Nosocomial Infection Surveillance (NNIS) system and found that the rate of nosocomial candidemia increased by almost 500% in large teaching hospitals and by 219% to 370% in small teaching hospitals and large non-teaching hospitals, respectively. This trend has continued into the 1990s. Edwards and Gaynes (2002), have recently reported that *Candida* species were the sixth most common nosocomial pathogens overall and the fourth most common bloodstream pathogens in US hospitals during the period 1990- 1992. The literature clearly illustrates the

clinical importance of *Candida* infections (Matthew *et al.,* 2005; Bala, 2004; Peter *et al.,* 2003; Elias *et al.,* 2002). As well as accounting for hematogenous infections in hospitalized patients, candidiasis is the most frequent mucosal yeast infection in patients suffering from HIV infection (Anna *et al.,* 2016). Epidemiologic studies have shown that intravascular catheters, antibiotic exposure, mucosal colonization, and neutropenia as significant risk factors for invasive Candidal infections (Peter *et al.,* 2003; Elias *et al.,* 2002; Matthew *et al.,* 2005; Bala, 2004; Seneviratne *et al.,* 2008). Currently, attention is being focused on the importance of non-albicans species as disease-causing agents of infection and to the development of an understanding of the likely potential reservoirs for-and modes of transmission of-nosocomial candidiasis. Over 100 species of *Candida* have been identified but only a few have been isolated from humans. *Candidia albicans* is the most common species isolated from clinical material and generally accounts for 50% to 70% or more of cases of invasive candidiasis (Matthew *et al.,* 2005; Traoré *et al.,* 2002; Bala, 2004; Elias *et al.,* 2002). Recent reports suggest that significant changes have occurred in the distribution of infections caused by *Candida* species other than C. *albicans* (Sarmad, 2009; Sally *et al.,* 2011). Although *C. albicans* is still the most frequent cause of fungemia and hematogenously disseminated candidiasis, an infective role has been shown for *Candida tropiealis, Candida glabrata, Candida parapsilosis, Candida krusei,* and *Candida lusitaniae* (Alberta *et al.,* 2014; Bunetel *et al.,* 2000), the emergence of such potentially pathogenic species of *Candida* other than C. *albicans* is clearly of interest. The aim of the work reported here was to isolate *Candida,* and other fungi, from the built environment and determine their survival of the former on tile- surfaces like those often found in health-care environments (computer keyboards, mobile telephones, sinks, used tooth brushes, vacuum cleaner dust, emitted by hand dryers and soles of shoes).

4.3. Isolation of yeasts from sinks.

4.3.1. Materials and Methods.

1) Isolation of samples and collection.

All yeasts used were isolated from sinks using sterile cotton swabs (Figure 4.1). The sinks examined were located as follows:

Sheffield University: Firth Court Building, Disability and dyslexia support service, The Alfred Denny Building, Information Commons Building, Students Union Building.

Other areas in Sheffield: Sheffield Train Station, local supermarket, local hospital, various private dwellings.

Figure 4.1: Sterile cotton swab used for sample collection.

2) Purification of isolates.

All samples were isolated from sinks and then streaked on media in petri dishes. The main medium used was Candida-identification Agar (Sigma-Aldrich) incubation was then at 25 °C for 3 to 5 days. The isolates were purified to single colonies and subjected to molecular identification.

Extraction of genomic DNA by using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit), Test samples preparation for PCR, DNA quantification, polymerase chain reaction (PCR), agarose gel electrophoresis and phylogenetic analysis, has been explained in Chapter 2**.**

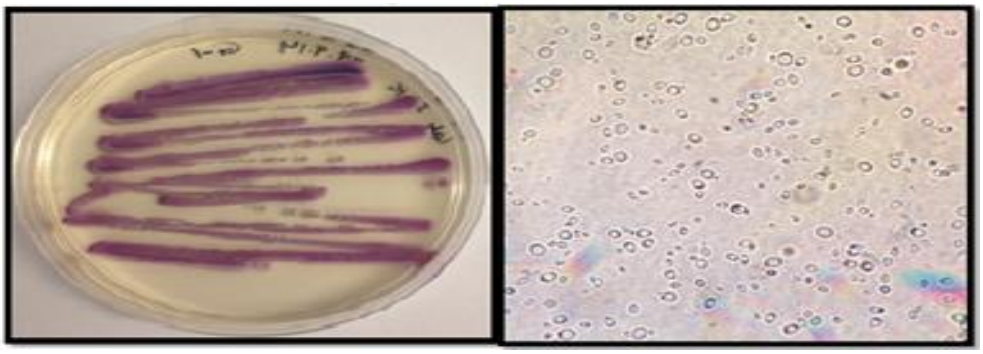
4.3.2. Results.

1) Isolation of Yeast from various sinks by cultivation on CIA medium.

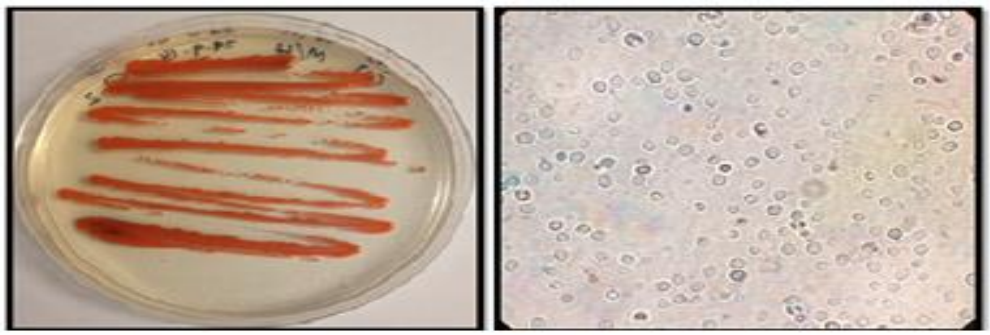
Candida species were isolated using CIA, This is a selective identification agar, which facilitates the rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* sp. This medium gives results within 48 hours and it is useful for rapid and presumptive identification of common yeasts in both the Mycology and Clinical Microbiology Laboratory (Perry, 1987; Rousselle *et al.*, 1994). A wide variety of colony types and colours of organisms were isolated on this medium. An indication of the yeast-nature of the isolate was given by the fact that the colonies were mucoid and not dry and filamentous which is typical of filamentous fungi.

2) Light microscope images.

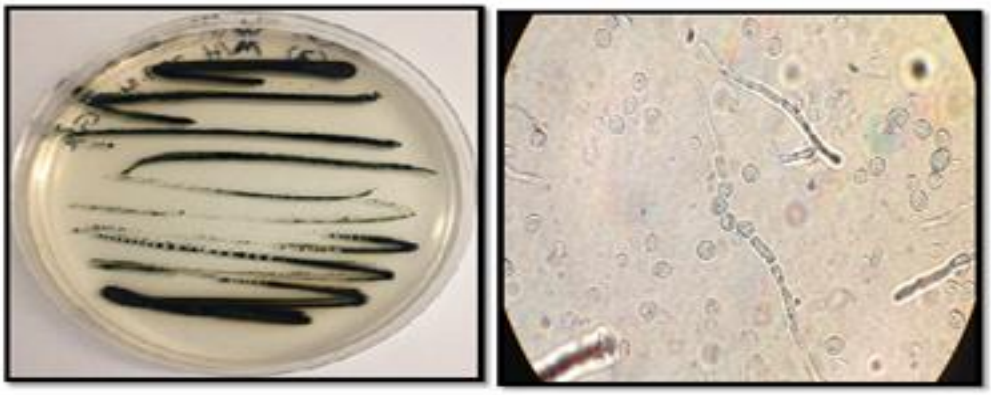
The isolates were examined under a light microscope (Figure 4.2) in order to confirm that they were yeasts, yeast-like fungi or dimorphic fungi undergoing a yeast-mycelium transition.



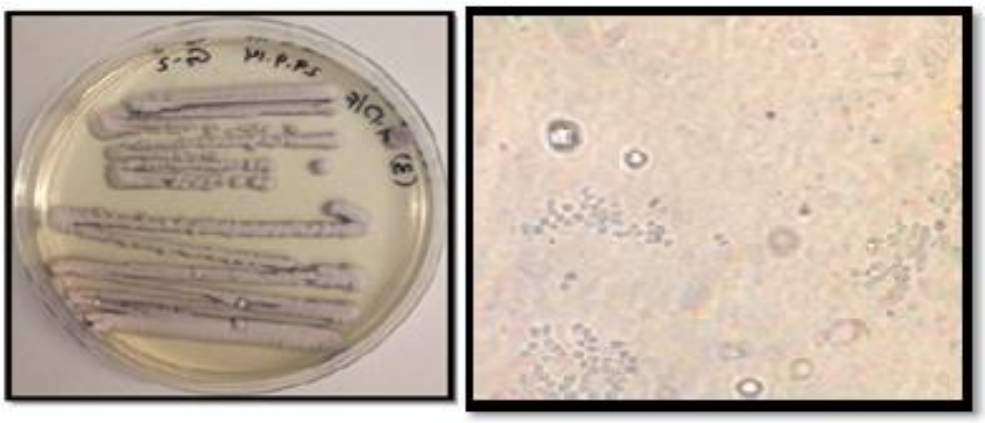
Meyerozyma guilliermondii.



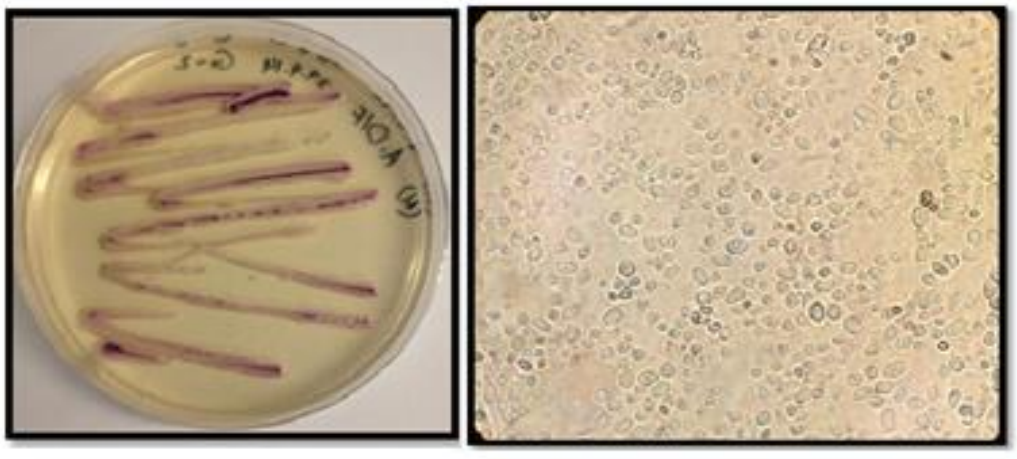
Rhodotorula mucilaginosa.



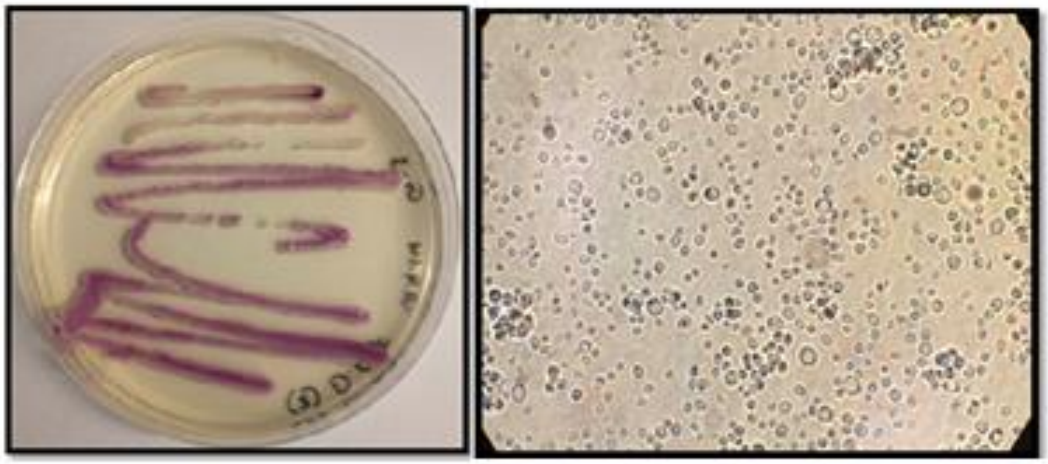
Exophiala phaeomuriformis.



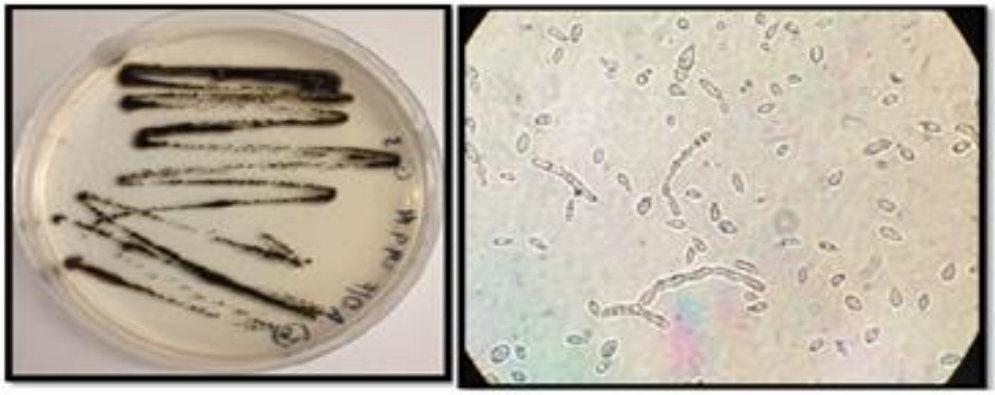
Fungal sp.



Candida parapsilosis.

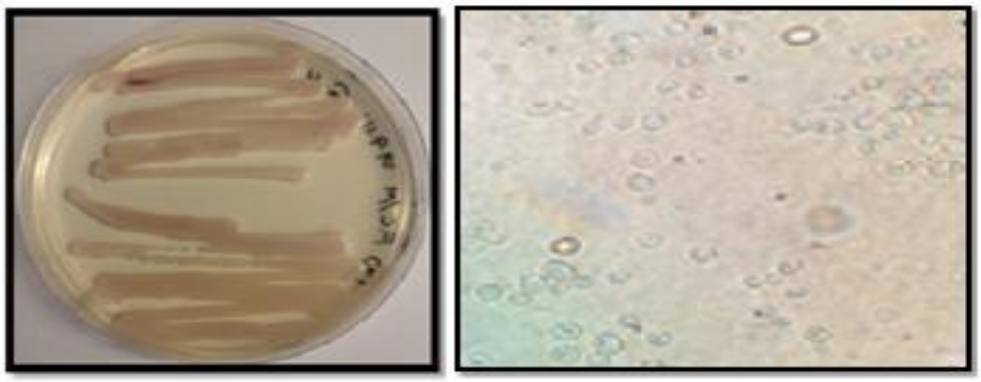


Pichia guilliermondii.

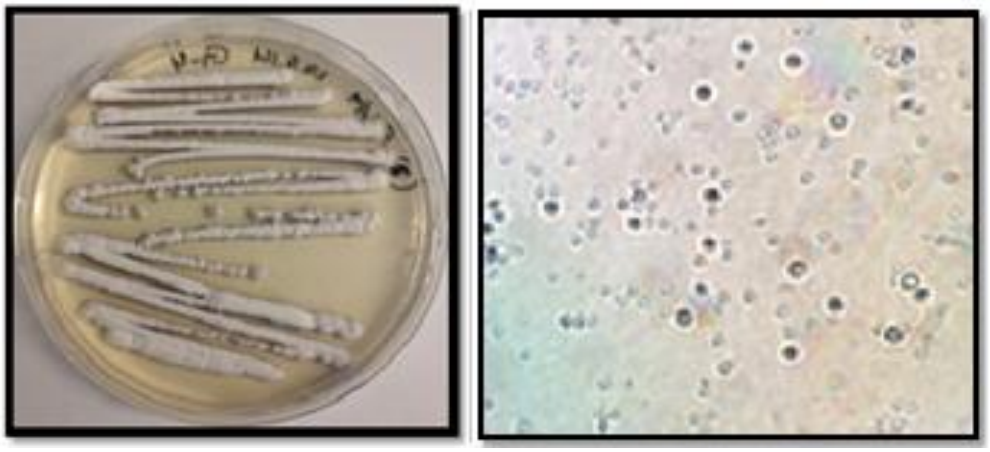


Exophiala lecanii.

Exophiala dermatitidis.



Paecilomyces lilacinus.



Yarrowia lipolytica.

Figure 4.2: CIA plates showing the result after streaking sterile cotton swabs from various sinks, also microscopy images shows the yeast species under the light microscope.

The images above show characteristic yeasts and filamentous fungi. Such light microscope investigation is valuable for providing back up to molecular identification methods. The isolates were next identified using 18SrRNA. The results are shown in Table 4.1.

Table 4.1: Yeasts and fungi isolated from various sinks.

Closest matches Identification	**Sequence Identity**	**Sequence ID**
Meyerozyma guilliermondii	100%	KM014576.1
Exophiala dermatitidis	99%	KJ740171.1
Uncultured fungus	100%	GU053979.1
Rhodotorula mucilaginosa	99%	KC182131.1
Exophiala phaeomuriformis	98%	KJ522802.1
Uncultured ascomycete	100%	AM901774.1
Fungal sp.	98%	JN802258.1
Candida parapsilosis	99%	KM113999.1
Yarrowia lipolytica	99%	KF851353.1
Pichia guilliermondii	100%	DQ663478.1
Exophiala dermatitidis	100%	NR_121268.1
Exophiala pisciphila	98%	AF050273.1
Exophiala lecanii-corni	100%	JX681040.1
Paecilomyces lilacinus	100%	FR751342.1

4.3.3. Discussion.

Microbial diversity has been defined to include genetic diversity by various counting techniques which include the plate count technique and the use of specific approaches such as 18SrRNA gene sequences (Nannipieri *et al.*, 2003). The molecular analysis of 18S rRNA gene has become dominant in research studies to examining the diversity of prokaryotic and eukaryotic microorganisms in the environment. The 18S rRNA gene has been chosen for use in phylogenetic analyses and eukaryote biodiversity (Meyer *et al.*, 2010), and 18S rRNA gene has become standard for examining and identifying eukaryotic microorganisms, such as fungi

(Hejazi *et al.*, 2010). A wide range of yeasts were isolated from the various sinks and identified; their characteristics in relation to pathogenicity are shown below:

Rhodotorula is an emerging opportunistic pathogen, notably in immunocompromised patients. A large number of cases of fungemia associated with catheters, *Meyerozyma guilliermondii* is widely isolated from a variety of natural environments and from fruit. It is the teleomorph of the opportunistic pathogen *Candida guilliermondii*, which causes about 2% of the human blood infections (Corte *et al.*, 2011). *Rhodotorula* infections are linked to underlying immunosuppression or cancer. The most common isolated risk factor associated with *Rhodotorul*a infection is however, the use of a central venous catheter, which has been was found in 83.4% of *Rhodotorula* fungemia (86 cases). *Rhodotorula mucilaginosa* is the most common species of fungemia, followed by *Rhodotorula glutinis*. *Rhodotorula species* in general have emerged as opportunistic pathogens that can infect susceptible patients. Most of the cases of infection due to *Rhodotorula* in humans were fungemia associated with central venous catheter (CVC) use. The most common underlying diseases included solid and haematologic malignancies in patients who were receiving corticosteroids and cytotoxic drugs (Worth and Goldani 2012), *Candida parapsilosis* is an emerging major human pathogen. *C. parapsilosis* is now one of the major causes of invasive Candidal disease. *C. parapsilosis* infections are especially associated with hyperalimentation solutions, prosthetic devices, and indwelling catheters, as well as the nosocomial spread of disease via the hands of health care workers. *Candida parapsilosis* is typically a commensal of human skin and forms biofilms on catheters and other implanted devices. Nosocomial spread is by hand carriage, and it persists in the hospital environment.

Chaetomium- exposure to this filamentous fungus can lead to classic allergy symptoms such as wheezing, runny eyes, and coughing and the fungus has also been linked with several cases of severe infections. *Chaetomium* can cause yeast infections of the toenail, and be a

threat to human health if it enters the bodies of immune-compromised individuals. It may also cause permanent neurological damage. A high rate of autoimmune diseases has been linked to exposure to this mold, and it has also been linked to certain forms of cancer. *Chaetomium globosum* is known to produce 2 toxins in moisture damaged buildings, chaetoglobosins A and C. These toxins have the potential to cause illness to building occupants. *Exophiala* species are common environmental filamentous fungi often associated with decaying wood and soil enriched with organic wastes. However, several species notably *E. jeanselmei, E. moniliae* and *E. spinifera,* are well documented human pathogens. Clinical manifestations include mycetoma (especially for *E. jeanselmei*), localized cutaneous infections, subcutaneous cysts, endocarditis and cerebral and disseminated infections.

Exophiala phaeomuriformis (a black yeast) is a rare causative agent of phaeohyphomycosis in cutaneous, subcutaneous and deep tissues and is responsible for 6.4% of infections caused by black yeasts. Infection usually occurs following skin abrasion or penetrating injuries (Alabaza *et al.,* 2009). *Exophiala haeomuriformis* can also cause corneal infection following eye exposure to contaminated water. People with cystic fibrosis are considered abnormally susceptible to *Exophiala* infections, including *E. phaeomuriformis*. It has been suggested that differences in the microbiota profiles of CF patients may be responsible for this predisposition. Treatment of *E. phaeomuriformis* involves a combination of surgical debridement and antifungal therapy. A range of antifungal agents including caspofungin, voriconazole, itraconazole, posaconazole, and amphotericin B are active against this species. Phaeohyphomycosis caused by *Exophiala* species has been reported in both normal and immunosuppressed patients. *Exophilia dermatitidis* is a dematiaceous fungus that is found in soil and dead plant material worldwide, and sometimes causes phaeohyphomycosis. This fungus plays a significant role as a respiratory pathogen in patients with cystic fibrosis. It is also cause of systemic or visceral infections, notably in patients with

compromised immunity. It is also known to cause local infections of the skin, which may spread and cause disseminated disease and fungemia; the latter is seen especially in immunocompromised patients. *Candida guilliermondii* (*Meyerozyma guilliermondii, Pichia guilliermondii*) is an uncommon species of *Candida* that is generally associated with onychomycosis and is rarely seen as a cause of invasive yeast infection. *Candida guilliermondii* is a component of the human microbiota, and is uncommonly isolated from patients as a pathogen. Owing to their lower frequency, infections caused by *C. guilliermondii* are relatively less studied, in comparison to infections caused by other *Candida* species. *Paecilomyces lilacinus* is a ubiquitous, saprobic filamentous fungus commonly isolated from soil, decaying vegetation, insects, nematodes and laboratory air (as contaminant), and is a cause of infection in man and other vertebrates. This species can colonize materials such as catheters and plastic implants and can contaminate antiseptic creams and lotions, causing infections in immunocompetent and immunocompromised patients. *Yarrowia lipolytica* strains are often isolated from dairy products such as cheeses, yoghurts and sausages. Strains have also been isolated from various environments, such as lipid-rich media (sewage, oil- polluted media) or marine and hypersaline environments. It has a marked ability to degrade proteins and lipids but has not been reported to be a pathogen. Most of the isolates obtained from sinks are potential pathogens, notably in relation to immunocompromised patients. While the isolates are generally unlikely to cause severe, life threatening infections in patients with a complete immunity, those suffering from AIDS, or who have their immunity compromised by other medical interventions may be at risk from these fungi. The presence in sinks of such yeasts, which are capable of inducing infection in such immunocompromised patients, is of obvious concern.

4.4. Isolation of *Candida* and filamentous fungi from computer keyboards.

Surface bio-contamination is a problem that contributes to outbreaks of community-acquired and nosocomial infections in the environment. Objects like computer keyboards and mouse can act as both reservoirs and transmitters of microbes including species of *Streptococcus epidermidis* and *Diptheroids*. *Trychophyton species, Aspergilus species* and *Candida albicans* and Bacilli. Most of these isolates are, not surprisingly found to be skin flora and are probably-dust associated. Routine cleaning of keyboards and mice or the use of transparent plastic covers together with hand hygiene is suggested as means of avoiding microbial contamination of keyboards and other computing devices. Contaminated personal computers have been implicated in transmission of methicillin-resistant *Staphylococcus aureus* to a nurse and they are often contaminated with Staphylococci and *Pseudomonas* spp. (Isaacs *et al.,* 1998). Keyboards have also been implicated in nosocomial *A. baumannii* infection in burn units (Neely *et al.,* 1999) and have with MRSA and *Enterobacter* spp. (Bures *et al.,* 2000). Multiple use cyber café's, in particular, are likely to act as a source of pathogen transfer (Goldmann, 2000).

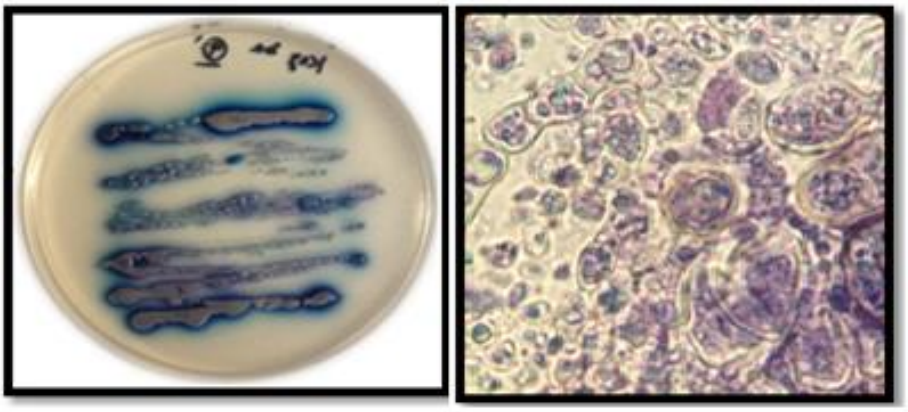
4.4.1. Materials and Methods.

All samples are isolated from computer keyboards using swabs (50 sample) then streaked on petri dishes containing CIA to determine yeast sp. then incubated at 25 °C for 3 to 5 days. Genomic DNA was extracted from each isolate was identified using 18S rRNA. The extraction of genomic DNA by using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit), Test samples preparation for PCR, DNA quantification, polymerase chain reaction (PCR), agarose gel electrophoresis and phylogenetic analysis, has been explained in Chapter 2**.**

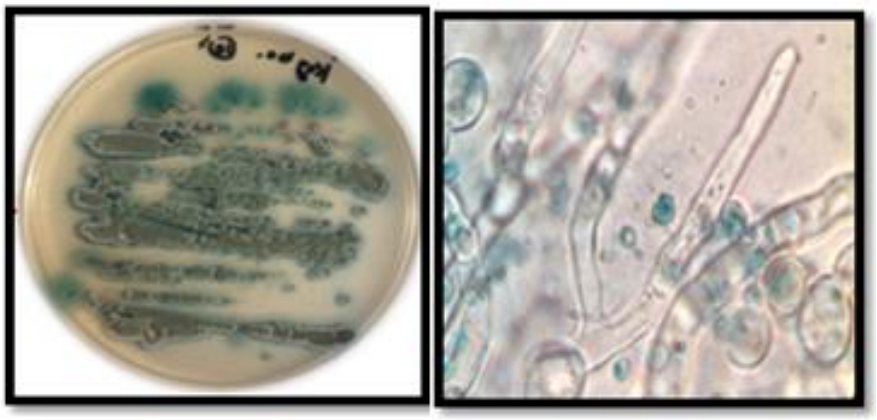
4.4.2. Results.

1) Light microscope images.

The isolates were examined under a light microscope (Figure 4.3) in order to establish their yeast, or filamentous fungal nature.



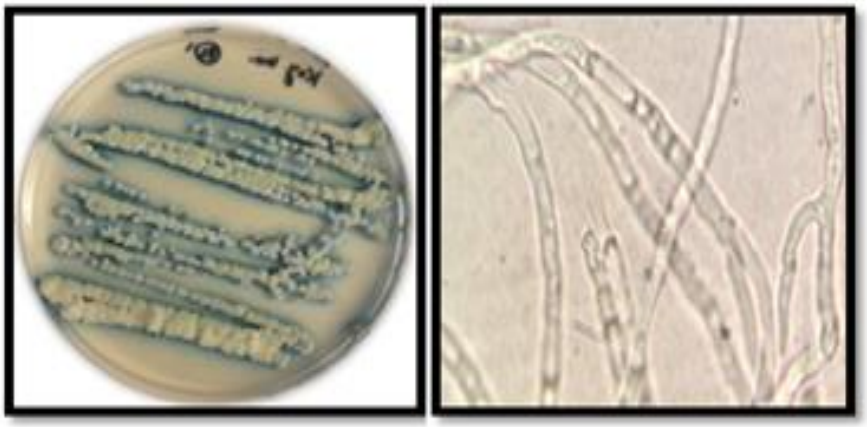
Aureobasidium pullulans.



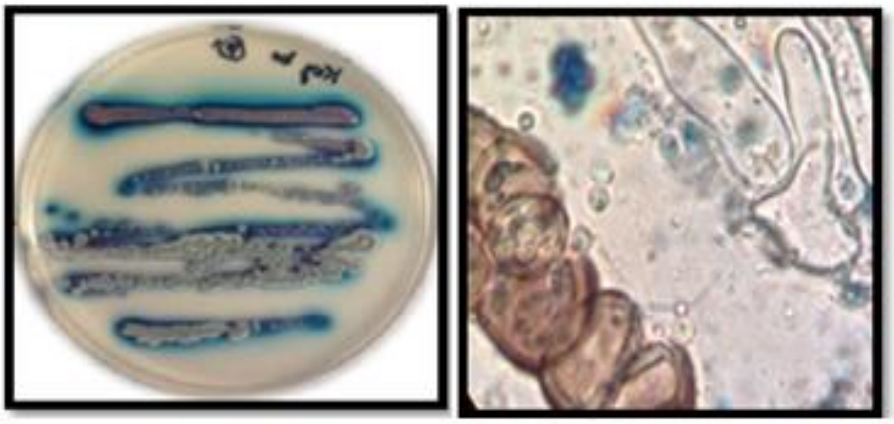
Alternaria alternate.

Penicillium brevicompactum.

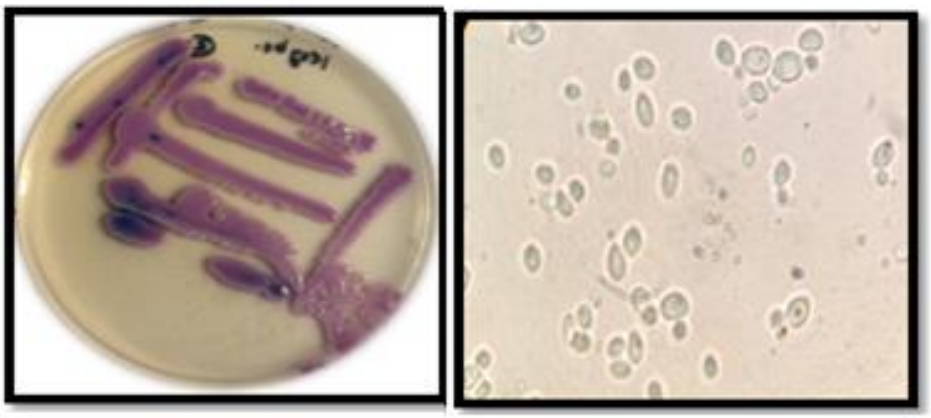
Penicillium citrinum.



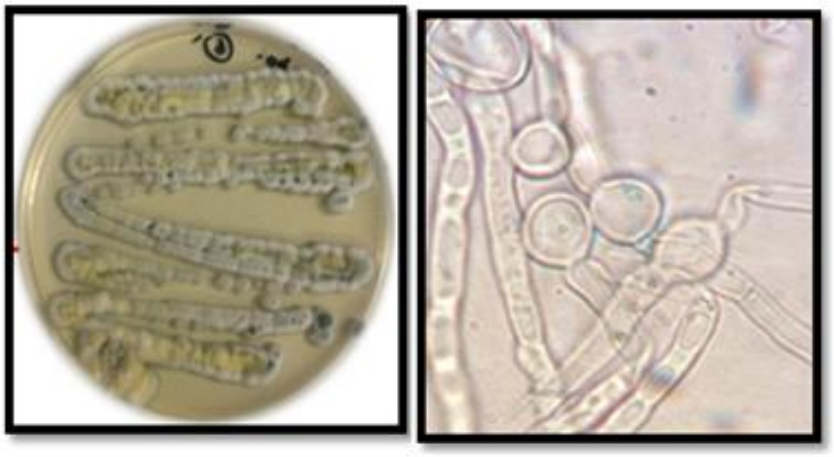
A yeast endophyte.



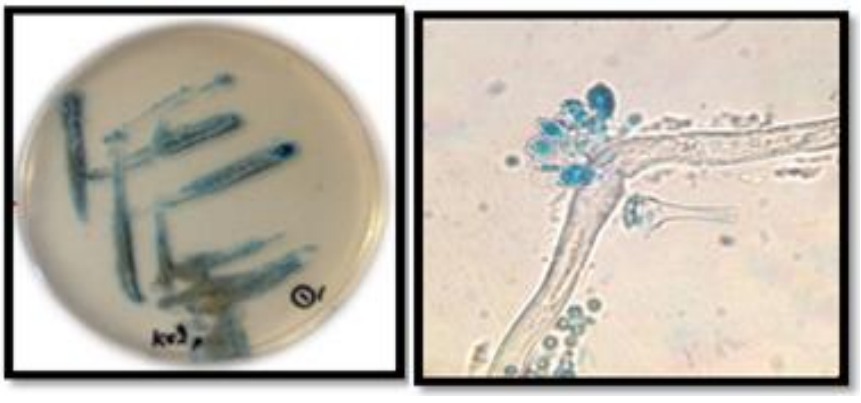
Aureobasidium pullulans.



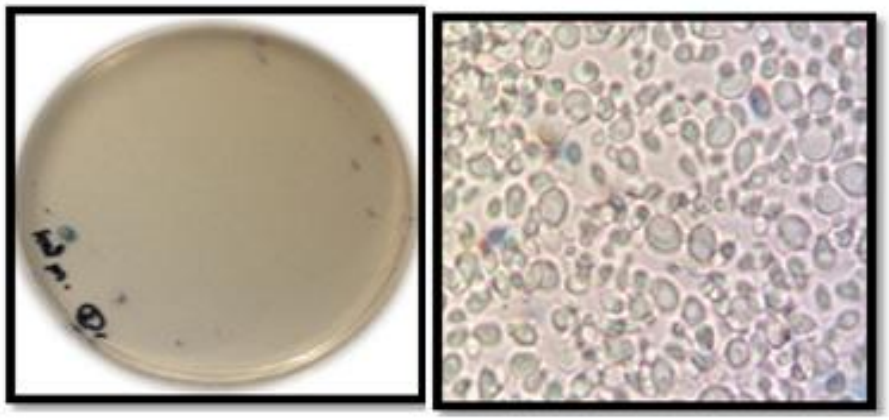
Penicillium olsonii.



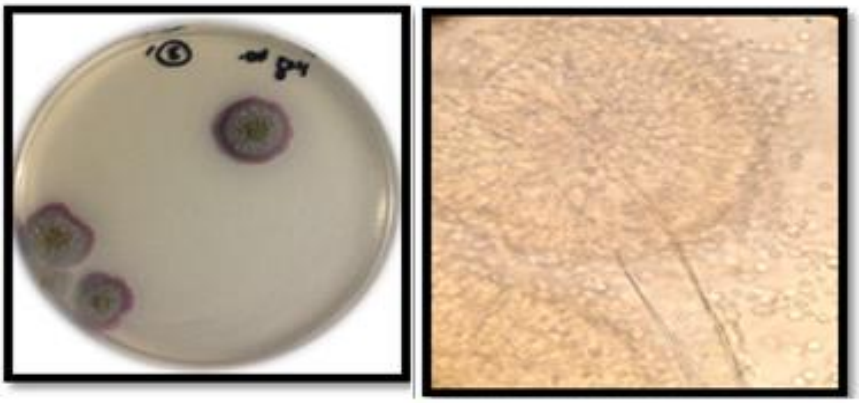
Penicillium chrysogenum.



Penicillium brevicompactum.



Meyerozyma guilliermondii.



Aspergillus niger.

Figure 4.3: CIA plates showing the result after streaking sterile cotton swabs from various computer keyboards; microscopy images are also shown.

The isolates were next identified using 18SrRNA. The results are shown in Table 4.2.

Table 4.2: Yeasts and filamentous fungi isolated from various computer keyboards.

Closest matches Identification	**Sequence Identity**	**Sequence ID**
Aureobasidium pullulans	99%	HQ267769.1
Alternaria alternate	99%	JN986771.1
Penicillium brevicompactum	99%	AY373897.1
Penicillium citrinum	100%	KM491892.1
A yeast endophyte	99%	KF436280.1
Aureobasidium pullulans	100%	KC897669.1
Penicillium olsonii.	100%	KM265447.1
Penicillium chrysogenum.	100%	KM396379.1
Penicillium brevicompactum.	99%	AY373897.1
Meyerozyma guilliermondii	100%	KP675395.1
Aspergillus niger	99%	JF436884.1

4.4.3. Discussion.

The results show that fungi, including yeasts were isolated from 30 computer key boards. Filamentous fungi predominated in terms of species diversity. The filamentous fungi isolated are all common air contaminants and could have been transferred to the keyboard surface by the operatives or by deposition from the air; none are pathogens. The yeast species include *Aureobasdium pullulans* which is generally described as being a dimorphic yeast-like fungus; it is again commonly distributed in soils, in the air and on leaf surfaces.

4.5. Isolation of *Candida* and other yeasts from mobile telephones.

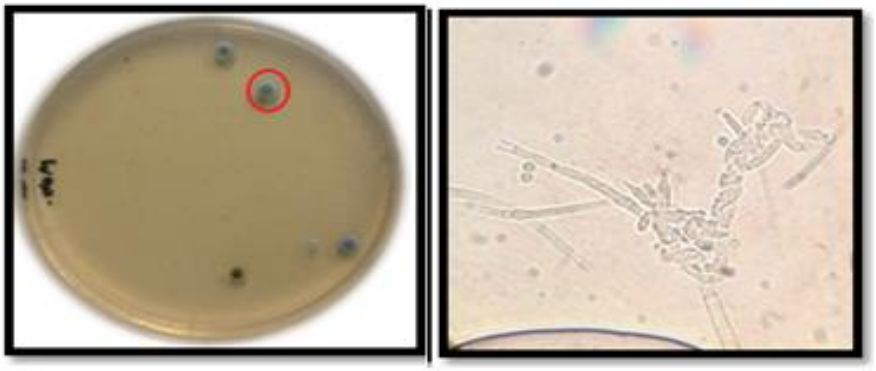
4.5.1. Materials and Methods.

Isolates were obtained from a variety of mobile telephones (30 sample) using CIA, incubation was at 25 °C for 3 to 5 days and the isolates were identified using 18S rRNA. Extraction of genomic DNA by using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit), Test samples preparation for PCR, DNA quantification, polymerase chain reaction (PCR), agarose gel electrophoresis and phylogenetic analysis, has been explained above.

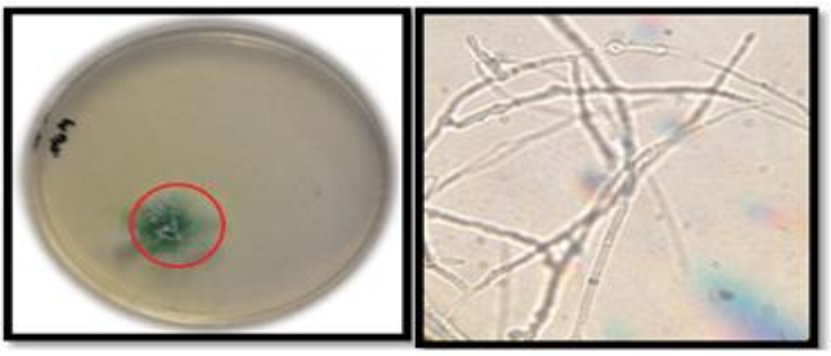
4.5.2. Results.

1) Light Microscope images.

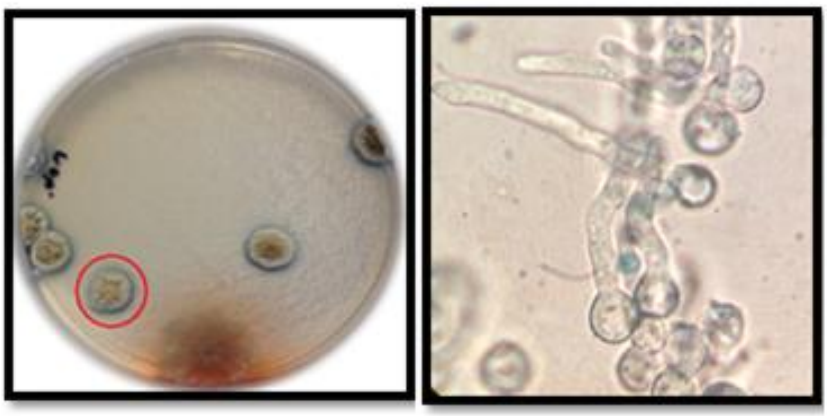
The isolates were examined under a light microscope (Figure 4.4) in order to determine if they are yeasts.



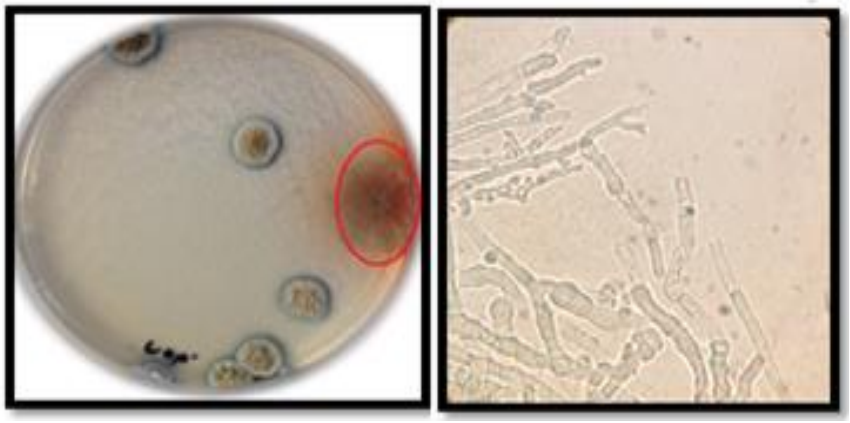
Yeast endophyte.



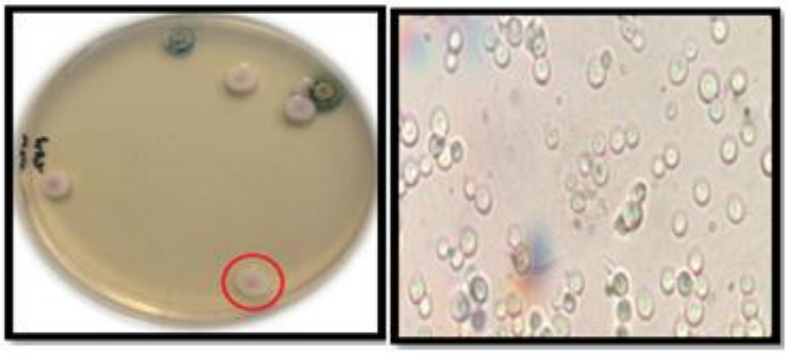
Chaetomium subaffine.



***Penicillium commune*.**



Chaetomium globosum.



Debaryomyces hansenii.

Figure 4.4: Isolates obtained from CIA plates showing the result after streaking sterile cotton swabs from various mobile devices; microscope images are also shown.

The isolates were next identified using 18SrRNA. The results are shown in Table 4.3.

Table 4.3: Yeasts and filamentous fungi isolated from various mobile phones

Closest matches Identification	Sequence Identity	Sequence ID
Yeast endophyte.	99%	KF436280.1
Chaetomium subaffine.	99%	JN209929.1
Penicillium commune.	99%	JN986756.1
Chaetomium globosum	99%	KM8226.1
Debaryomyces hansenii	100%	KM521205.1

4.5.3. Discussion.

As with the keyboard studies, most of the isolates were filamentous fungi, all of which are common air contaminants and none are pathogens. A species of *Debaryomyces* was the only yeast isolated.

4.6. Isolation of *Candida* and yeasts from vacuum cleaner dust samples obtained from carpets, textiles and upholstered furniture.

4.6.1. Materials and Methods.

Dust samples were obtained from a variety of different vacuum cleaners (20 sample) for use to determine the yeast content of the waste. The dust was placed in the surface of CIA and then incubation at 25 °C for 3 to 5 days. Extraction of genomic DNA by using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit), Test samples preparation for PCR, DNA quantification, polymerase chain reaction (PCR), agarose gel electrophoresis and phylogenetic analysis, has been explained above**.**

4.6.2. Results

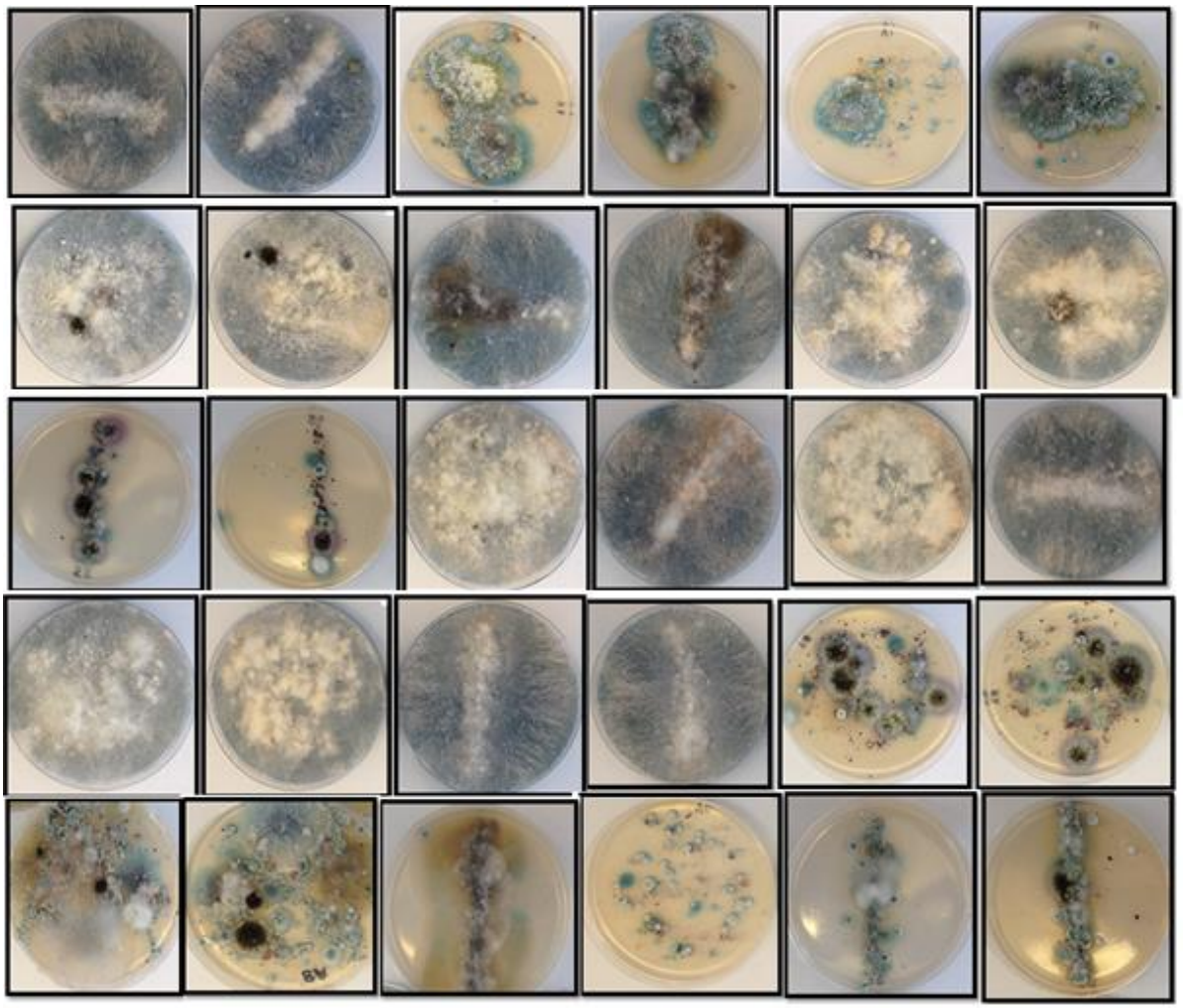
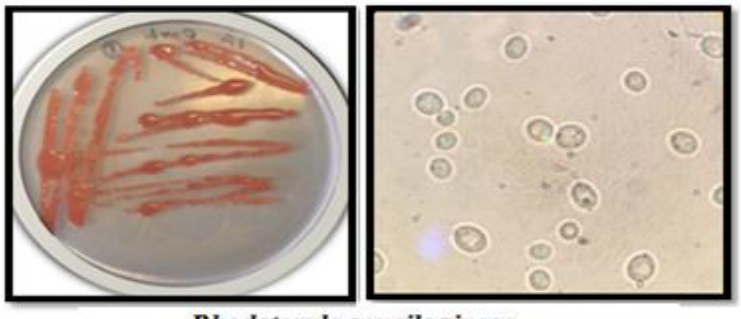


Figure 4.5: Fungi and yeasts isolated on CIA from dust samples obtained using various vacuum cleaners.

1) Light microscope images.

In order to confirm that they were yeasts, the isolates were examined under a light microscope (Figure 4.6).



Rhodotorula mucilaginosa

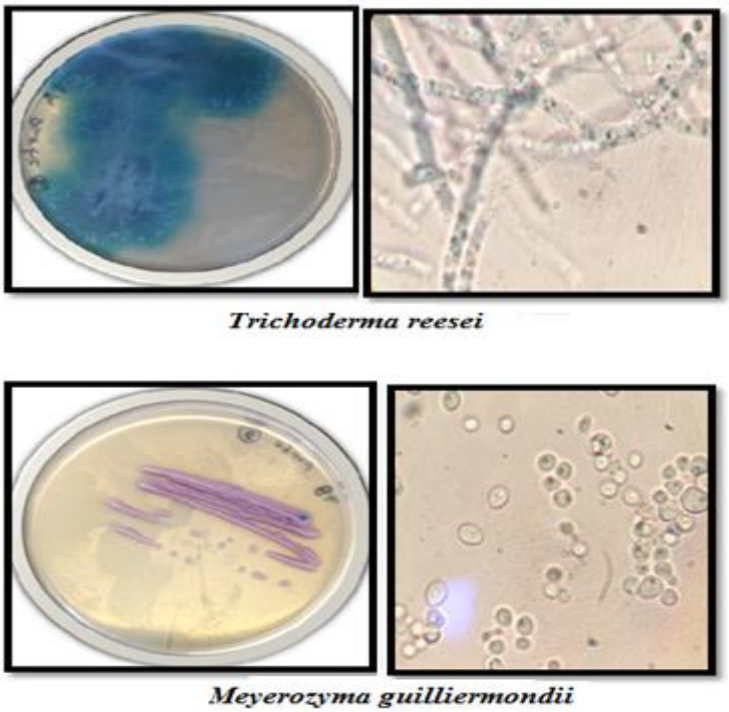


Figure 4.6: Microscopy images shows examined yeast sp. under the microscope.

The isolates were next identified using 18SrRNA. The results are shown in Table 4.4.

Table 4.4: Yeasts and fungi isolated from dust samples of various vacuum cleaners

Closest matches Identification	**Sequence Identity**	**Sequence ID**
Rhodotorula mucilaginosa	100%	KC816558.1
Trichoderma reesei	100%	KP216890.1
Meyerozyma guilliermondii	100%	KP132430.1

4.6.3. Discussion.

A large number of filamentous fungi and yeast colonies were isolated from vacuum cleaner dust. Of these, only three isolates were confirmed by molecular analysis, namely two yeasts and the filamentous fungus *Trichoderma reesei*. Both yeasts are not regarded as pathogens in the normal sense, but are likely to cause infections in immunocompromised patients; their presence in carpets (i.e. vacuum cleaner dust) is therefore of potential concern in certain health care settings.

4.7. Isolation of *Candida* and filamentous fungi from the soles of shoes.

The transfer of microorganisms from the soles of shoes to floors and other surfaces within health care environments is an obvious potential infectious hazard, as many potential pathogens (again notably in relation to immunocompromised patients) can be carried in this way from environmental sources such as soils and cat and dog faeces. The wearing of shoe-cover-alls is generally a requirement for entry into intensive care areas of hospitals, but it is regarded as too troublesome (or not necessary) to make everyone who enters a medical facility undertake this precaution. As a result, there is little doubt that microbes are transferred from outside of medical facilities to the wards etc. As a result it is obviously important that the floors of medical facilities be regularly, and thoroughly, disinfected.

4.7.1. Materials and Methods.

All yeasts were isolated from various shoes bottom (30 sample) by the use of sterile cotton swabs which were then placed onto the surface of CIA and then incubated at 25 °C for 3 to 5 days.

Extraction of genomic DNA by using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit), Test samples preparation for PCR, DNA quantification, polymerase chain reaction (PCR), agarose gel electrophoresis and phylogenetic analysis, has been given above**.**

4.7.2. Results.

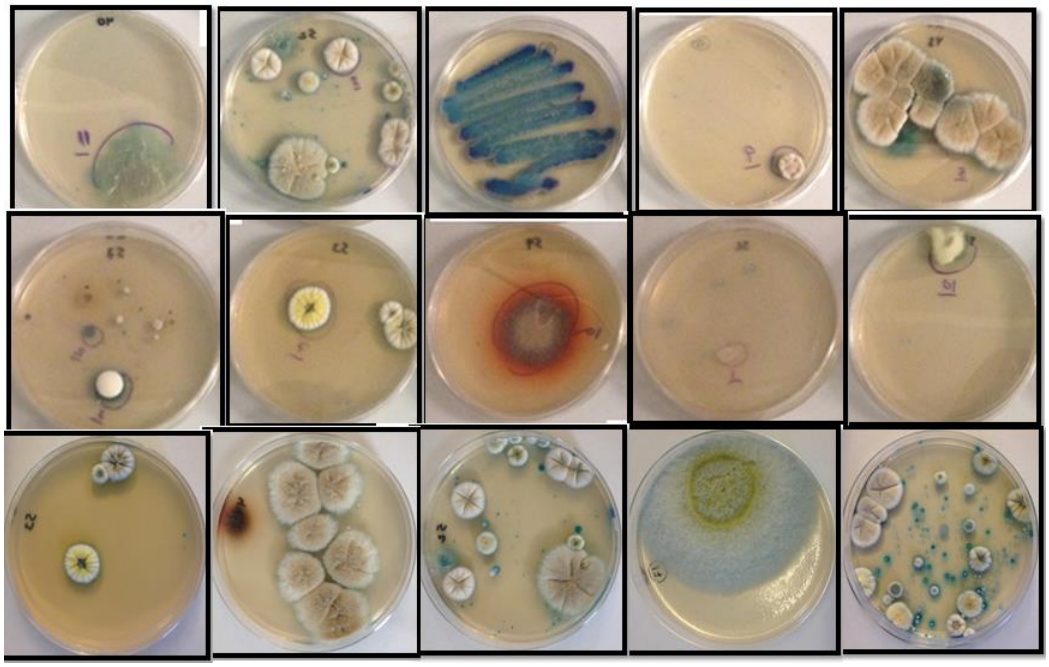
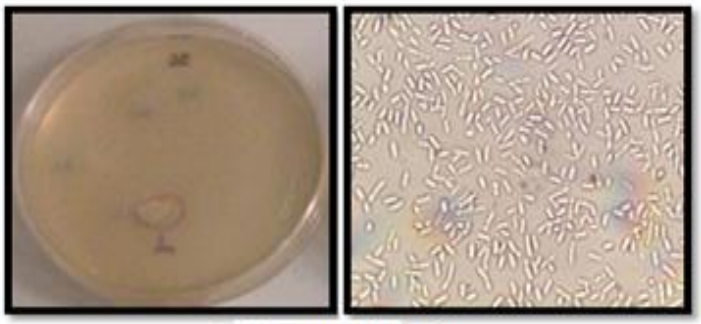


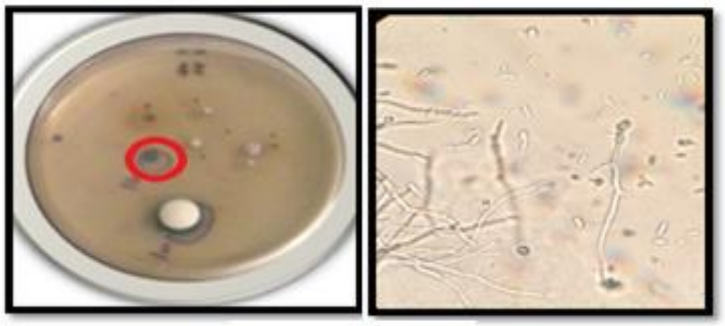
Figure 4.7: Fungi and yeasts obtained from the soles of shoes using CIA.

1) Light microscope images.

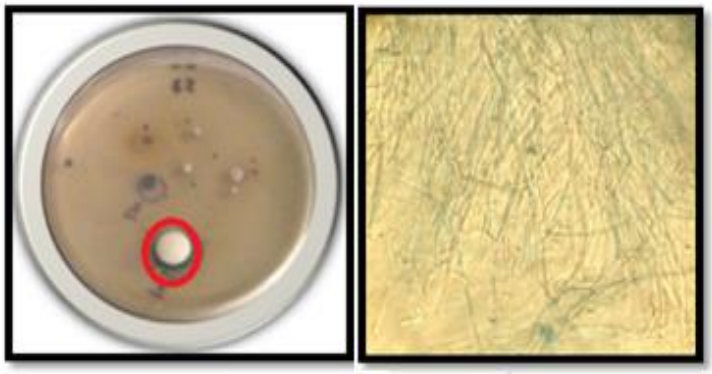
The isolates were examined under a light microscope (Figure 4.8) in order to determine if they are yeasts.



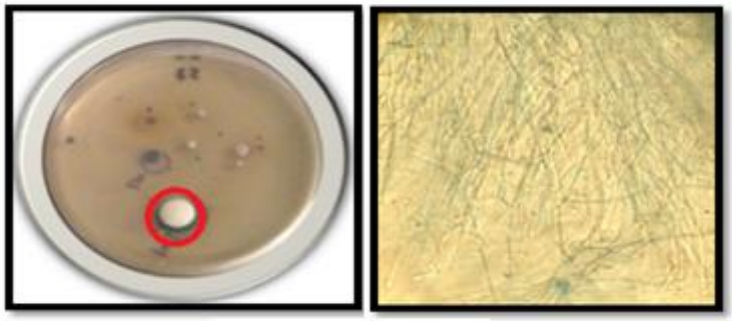
Arthrographis kalrae



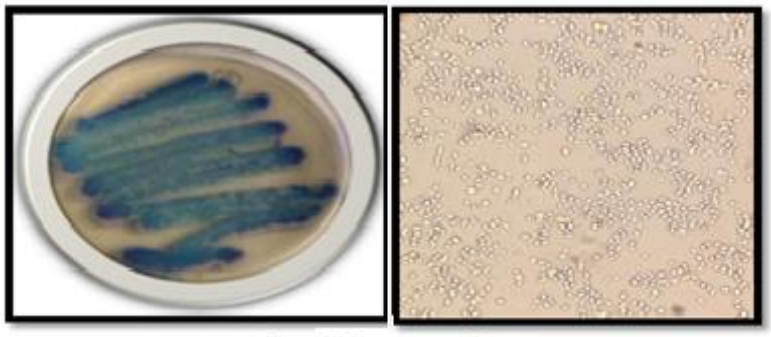
Lecythophora sp



Beauveria bassiana

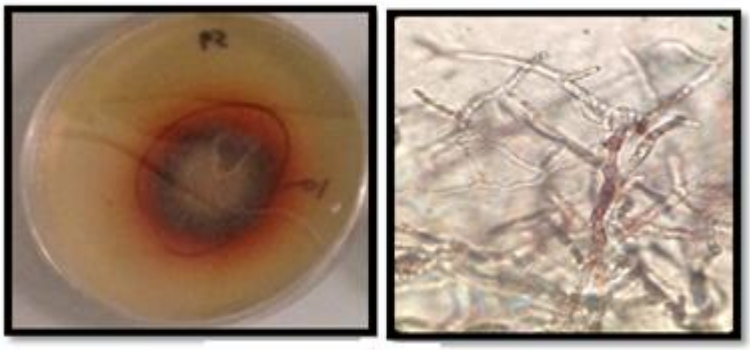


Beauveria bassiana

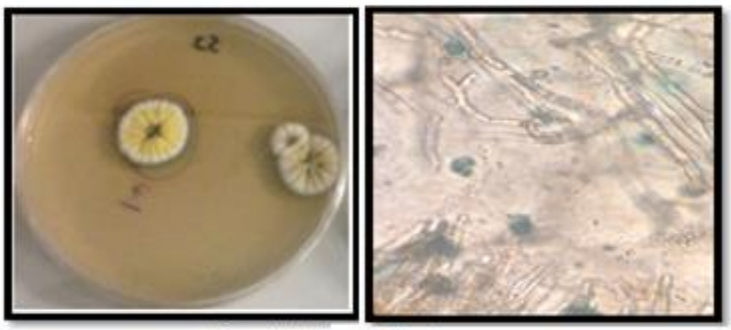


Candida catenulate

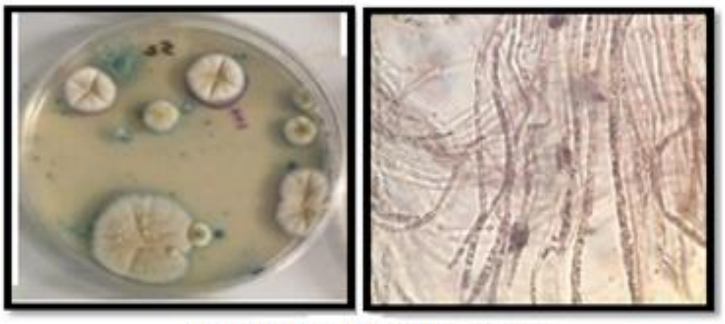
Aspergillus fumigates



Chaetomium globosum



Penicillium flavigenum



Penicillium simplicissimum

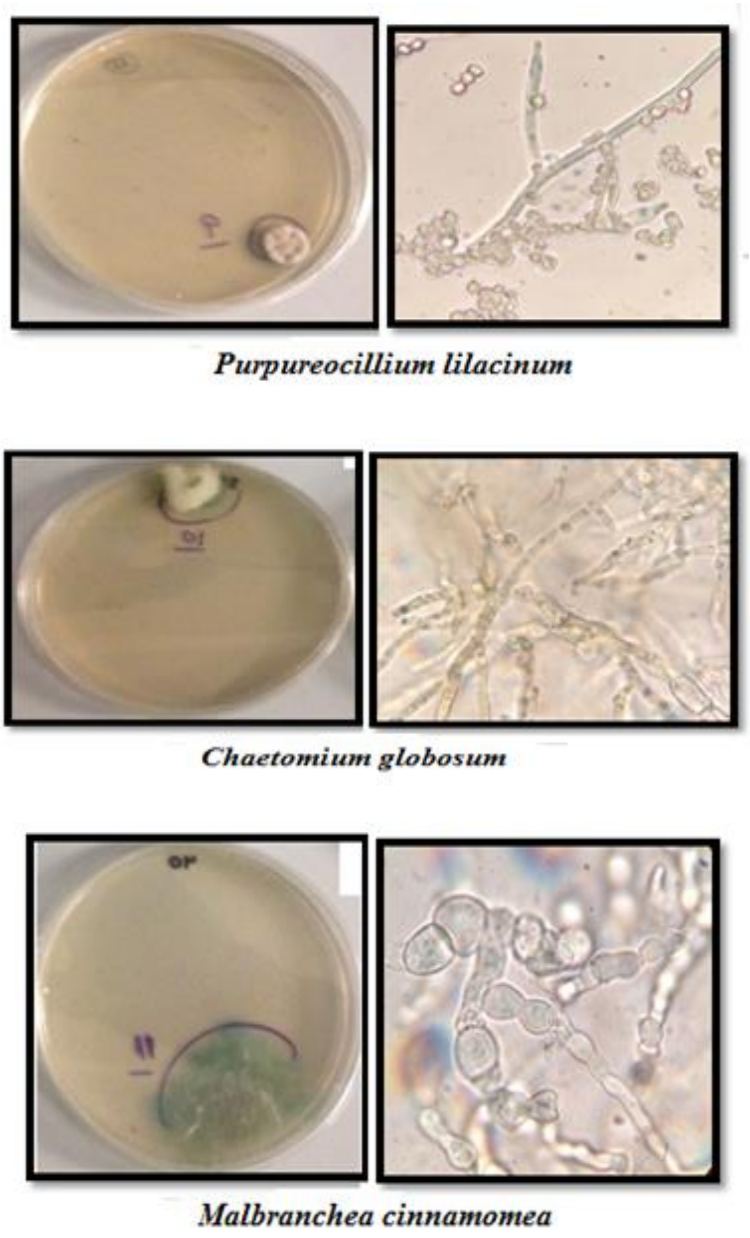


Figure 4.8: Microscope images of one yeast and filamentous fungi isolates.

The isolates were next identified using 18SrRNA. The results are shown in Table 4.5.

Table 4.5: Yeasts and fungi isolated from the soles of various shoes.

Closest matches Identification	**Sequence Identity**	**Sequence ID**
Arthrographis kalrae	100%	KP131545.1
Lecythophora sp	99%	LN825695.1
Beauveria bassiana	99%	EU334678.1
Candida catenulate	100%	KP131684.1
Aspergillus fumigatus	99%	KP972566.1
Chaetomium globosum	100%	KR012911.1
Penicillium flavigenum	100%	KR261456.1
Penicillium simplicissimum	99%	JN903543.1
Purpureocillium lilacinum	100%	KP308838.1
Chaetomium globosum	99%	KR012922.1
Malbranchea cinnamomea	99%	GU966515.1

4.7.3. Discussion.

The results show that a wide range of filamentous fungi could be isolated from the soles of shoes worn in normal use; in contrast, one yeast species were isolated. The apparent absence of yeasts can be explained by fact that this environment is likely to be generally dry and lack high levels of easily available carbon-rich nutrients. In addition, unlike filamentous fungi, yeasts do not produce resistant spores, which can withstand the relatively harsh conditions which exist on shoe soles. The results do however show that visitors to hospitals and other health care settings will carry spores of numerous filamentous fungi on the soles of their shoes (Lai, 2001; Mehta, 1990). While none of the fungal isolates are major pathogens, some are likely to be a risk factor for immunocompromised patients. This reality is recognized by

the fact that in critical care facilities visitors and staff are made to wear flexible plastic overshoes to prevent microbial contamination from outside (Falvey and Streifel 2007; Lai, 2001; Mehta, 1990).

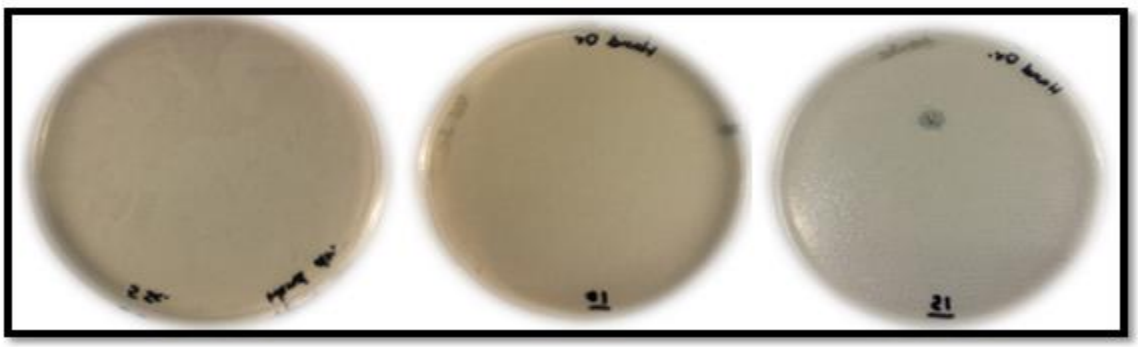
4.8. Transfer of yeasts to the air by hand dryers.

Redway *et al.*, (2008) showed that microorganisms are spread into the local environment when hand air dryers are used, much more so than when paper towels are used, thereby leading to an increased the risk of pathogen contamination of the environment from the hands of users; a similar conclusion was arrived at by Blackmore (1989).

4.8.1. Materials and Methods.

CIA plates were exposed for various times to the exhaust air from a hot hand dryer, for time periods ranging from 5 seconds to 1 minute.

All samples were isolated after exposed for various times to the hot air dryers emissions on media in petri dishes. The main medium used was Candida-identification Agar incubation was then at 25 °C for 3 to 5 days. The isolates were purified to single colonies and subjected to molecular identification. Extraction of genomic DNA was achieved using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit), Test samples preparation for PCR, DNA quantification, polymerase chain reaction (PCR), agarose gel electrophoresis and phylogenetic analysis, was determined as described above.



5 Seconds **10 Seconds** **15 Seconds**
(***Bjerkandera adusta***)

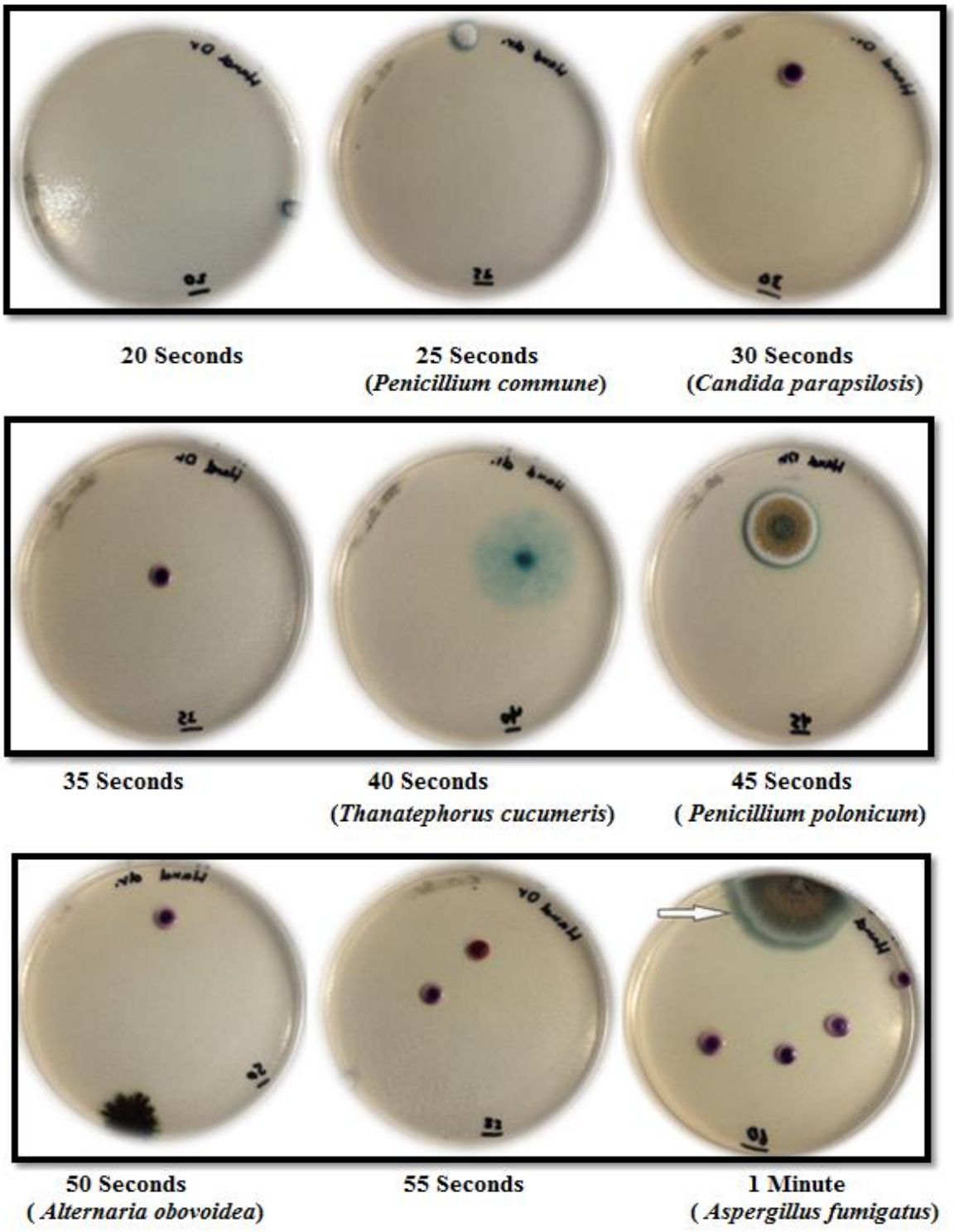
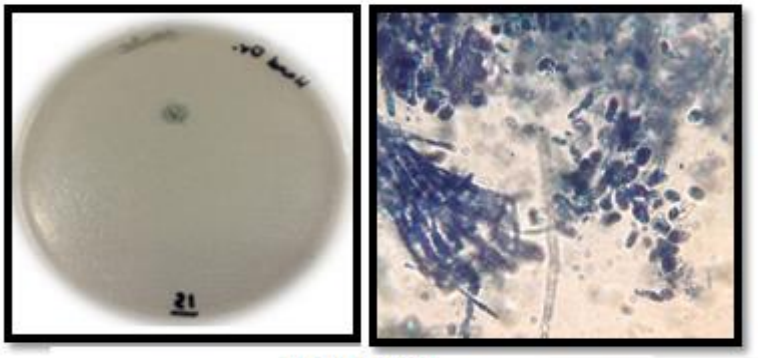


Figure 4.9: Isolates obtained following exposure to the air stream for various time periods.

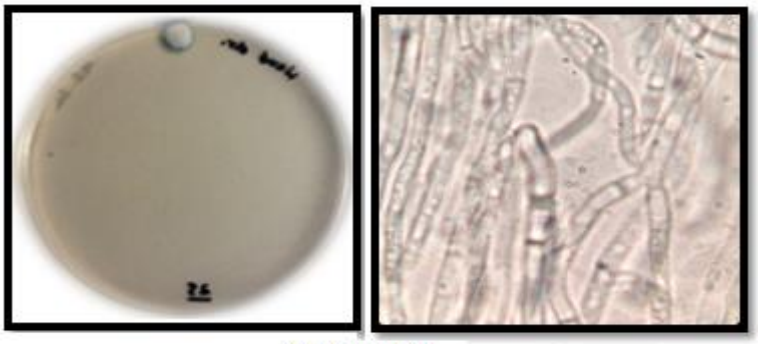
4.8.2. Results.

1) Light microscope images.

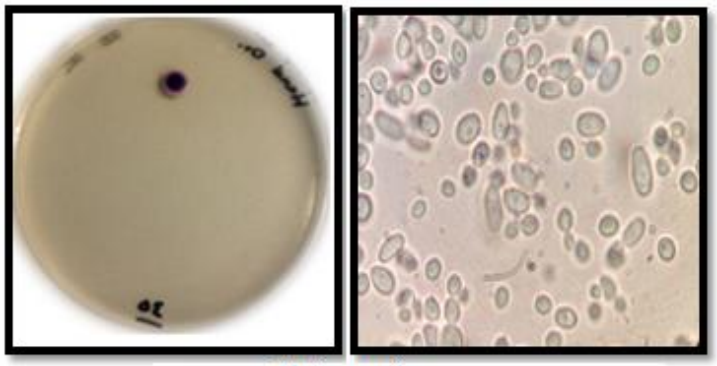
The isolates were examined under a light microscope (Figure 4.10) in order to determine which are yeast species.



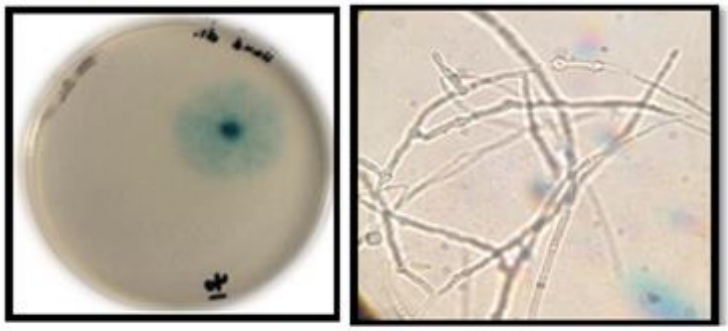
15 Seconds.
(*Bjerkandera adusta*)



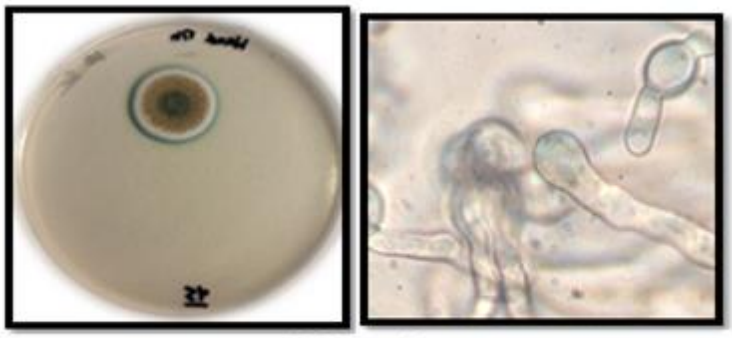
25 Seconds.
(*Penicillium commune*)



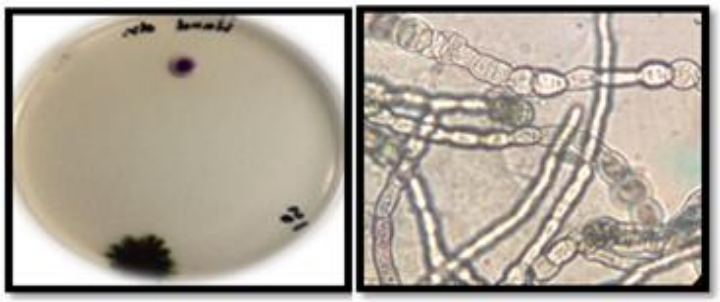
30 Seconds.
(*Candida parapsilosis*)



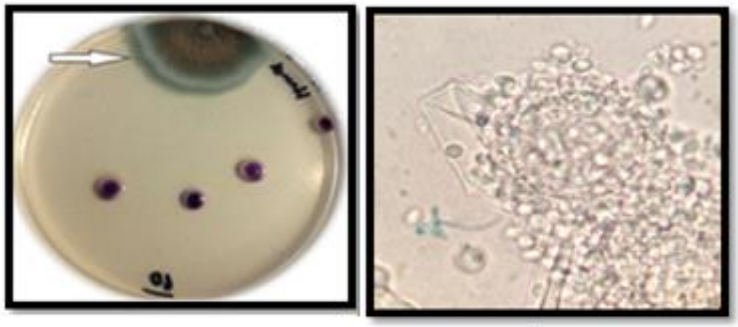
40 Seconds.
(*Thanatephorus cucumeris*)



45 Seconds.
(*Penicillium polonicum*)



50 Seconds.
(*Alternaria obovoidea*)



1 Minute
(*Aspergillus fumigatus*)

Figure 4.10: Microscope images of the isolates.

The isolates were next identified using 18SrRNA. The results are shown in Table 4.6.

Table 4.6: Yeasts and other fungi isolated from various hand warm air dryers.

Closest matches Identification	**Sequence Identity**	**Sequence ID**
Bjerkandera adusta	99%	KF681360.1
Penicillium commune	99%	KF938402.1
Candida parapsilosis	99%	KM113999.1
Thanatephorus cucumeris	100%	KF381087.1
Penicillium polonicum	99%	KF848936.1
Alternaria obovoidea	100%	KC466541.1
Aspergillus fumigatus	100%	KP412245.1

4.8.3. Discussion.

From these results, it is clear that hand dryers distribute filamentous fungi into the surrounding air. Non-pathogenic, filamentous fungi, predominate the isolate-list, *Candida parapsilosis* being the only yeast species isolated. The fact that filamentous fungi produce airborne, resistant spores undoubtedly accounts for their predominance in hand dryer emissions. As has been mentioned elsewhere in this Thesis, *Candida parapsilosis* is a potential problem for immunocompromised patients, and the same might apply to some of the filamentous fungi which have been isolated here. The use of hand dryers has become increasingly popular over the last few years and for reasons of cost, these machines have largely replaced traditional fabric and paper towels (Huang *et al*., 2012). While this should not present a health problem for most hospital visitors and patients, again the situation is likely to be different for immunocompromised patients, so it so clearly undesirable that electrical hand dryers be used in hospitals and other health care settings. The general

consensus from the literature is that paper towels are the least contaminating means of hand drying for use in health care settings (Best, *et al*, 2014; Huang *et al.*, 2012).

4.9. General Discussion.

Despite the importance of many species of *Candida* as human pathogens, little is known about their ability to survive on animate and inanimate surfaces. Traore et al. (2002) studied the survival of one clinical isolate each of *Candida albicans* and *C. parapsilosis* on glass and stainless steel and two fabrics namely100% cotton and a blend of 50% cotton and 50% polyester. The survival of *C. albicans* was also assessed on human skin, using the fingerpads of adult volunteers. When dried on glass and stainless steel *C. albicans* and *C. parapsilosis* respectively remained viable for at least three and 14 days; while both could survive for at least 14 days on both fabrics. On the skin, 20% of the viable *C. albicans* remained detectable one hour post-inoculation. Infections due to various species of *Candida* are becoming increasingly common (Jarvis 1995; Pfaller 1995; Pfaller *et al.,* 1998); such infections account for 8±15% and 9á3% of the cases of nosocomial septicemia in the US and Europe (Flanagan and Barnes 1998), respectively. Immunocompromised patients are particularly vulnerable (Jarvis 1995). Candida species are the fourth most common nosocomial pathogen in intensive care units (Pfaller 1995). Patients with haematological malignancies, 20±50% show invasive yeast infections at autopsy (Ascioglu *et al.,* 2000) and for neutropenic patients the colonization of the gastrointestinal tract or the oral cavity by species of *Candida* can increase the risk of systemic candida infection (Ascioglu *et al.,* 2000).

Nosocomial dissemination of *Candida* species can take routes similar to other nosocomial pathogens, notably by transient carriage on the hands of the staff (Sanchez *et al.*, 1993; Vazquez *et al.* 1993, 1998). *C. albicans* and *C. parapsilosis*, suspended in a soil load and dried on to carriers of porous or non-porous inanimate materials, were found to remain viable

for several days under ambient conditions; such survival was similar to that of Gram-positive bacteria including *Staphylococcus aureus* or *enterococci* (Neely and Maley 2000). *C. parapsilosis* can survive on hard environmental surfaces much more effectively than can *C. albicans*. After seven days, *C. albicans* was not recoverable from the non-porous inanimate carriers, while *C. parapsilosis* was still viable after14 days. On fabrics, the survival difference between *C. albicans* and *C. parapsilopsis* was less marked. Studies have shown that nurses' uniforms could frequently become contaminated with multi-resistant *Staphylococcus aureus* and act as a recervoir for cross contamination (Boyce *et al.,* 1997); such vehicles are likely to be involved in the spread of *Candida*. As a rule, survivability of nosocomial pathogens on environmental surfaces is inversely proportional to air temperature. Therefore, the ability of *Candida* to retain viability on porous and non-porous inanimate surfaces would be expected to be higher at refrigeration temperatures (4±10°C). While conversely, pathogenic yeasts might be expected to become inactivated faster at temperatures normally found in buildings with climate control, circa 22 degrees (Ribera *et al.,* 1994).These results show that porous and non-porous surfaces as well as hands can act as major vehicles for spreading *Candida* in health settings.

CHAPTER 5

ISOLATION OF YEASTS AND FILAMENTOUS FUNGI FROM USED TOOTHBRUSHES AND DETERMINATION OF ANTI - YEAST POTENTIAL OF TOOTHPASTES.

5.1. Introduction.

Although humans are sterile at birth, a great variety of microbes develops during the first day and includes species of *Streptococcus, Staphylococcus, Neisseria, Candida, Lactobacillus, Veillonella* and coliforms (McCarthy et al., 1965; Socransky and Manganiello 1971). However, *Streptococci* the primary etiological agent of human dental is found only after dental eruption because of its need to develop on hard surfaces. Catalanotto 1975; Fujiwara 1991; Glass, (Glass 1992) showed that microbes adhere to and reproduce on used toothbrushes and have the ability to transmit pathogens responsible for both local and systemic diseases. He showed that viruses like herpes simplex type I can survive for 48 h on toothbrushes that had been artificially air-dried and for 7 days plus on moist toothbrushes. Glass and Lare (1996) suggested that toothbrushes are an important means of pathogen transmission, especially to those receiving organ transplantation or suffering from immunological depression. According to the literature, under normal storage conditions, toothbrushes can be a source and a vector for transmission or re-infection of diseases including periodonto-pathogenic microorganisms, 18 as well as coliforms from the bathroom environment (Verran 1996). Toothbrushes provide obvious object on which oral microbes including pathogens could potentially survive and be passed on. While it is of course unlikely that toothbrushes will be intentionally shared amongst families and individual patients in hospitals the potential exists for accidental contamination-transfer. Perhaps more importantly, is the fact that pathogens could re-infect patients from toothbrushes at the point where they

become immunocompromised, thereby recycling pathogens, including antibiotic resistant species. The fact that toothbrushes are often exposed to sunlight and nearly always to drying is likely to produce conditions which are not conducive to pathogen survival. Toothbrushes rapidly become heavily contaminated with oral microorganisms including bacteria, viruses and fungi. These include dental pathogens such as Streptococcus mutans, organisms associated with periodontal disease and opportunistic yeast pathogens such as Candida albicans (Sammons et al., 2004). In addition, organisms not normally associated with the oral flora have been isolated from toothbrushes, including Enterobacteria and Pseudomonads. Toothbrushes are therefore considered to be potential sources of both oral and systemic infection and re-infection (Sammons et al., 2004). The presence and increase in number of these microorganisms can cause a significant risk of dissemination for certain individuals at risk, notably such as immunocompromised patients such as those receiving skin grafts or organ donation and those patients suffering from diabetic, cardiovascular disease; such organisms may also cause major problems during pregnancy (Bunete et al., 2000). The aim of the work described in this section was to determine if tooth brushes undergoing regular household use are contaminated with yeasts.

5.1.1. Materials and Methods.

Fifteen toothbrushes were collected from volunteers aged 5 to 45 years. The toothbrushes were transported to the laboratory in a sterile polythene bag sealed with a rubber band. Brushes were processed within 12 h by a method modified from that described by Sammons *et al*., (2004). The handle was cut off using a rotary saw and the head of the brush was retained in the bag to avoid contamination. Each brush head was then subjected to soaking in 10ml of sterile water, for 20 min and manual swabbing to dislodge persistently adherent yeast. The resulting yeast suspension was serially diluted and 0.1ml aliquots plated onto CIA,

to select yeasts. Plates were incubated aerobically at 25 ^{o}C for 3-5 days. Total viable counts were estimated from the numbers of colonies on the inoculated plates. Colony color, morphology was performed on a representative of each colony morphotype then, the colonies were identified.

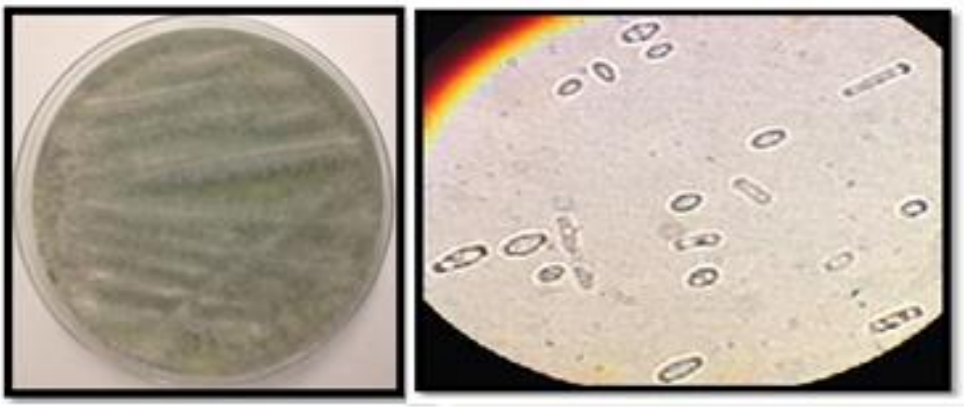
Extraction of genomic DNA by using (Norgen's Fungi/Yeast Genomic DNA Isolation Kit), Test samples preparation for PCR, DNA quantification, polymerase chain reaction (PCR), agarose gel electrophoresis and phylogenetic analysis, has been explained above**.**

5.1.2. Results.

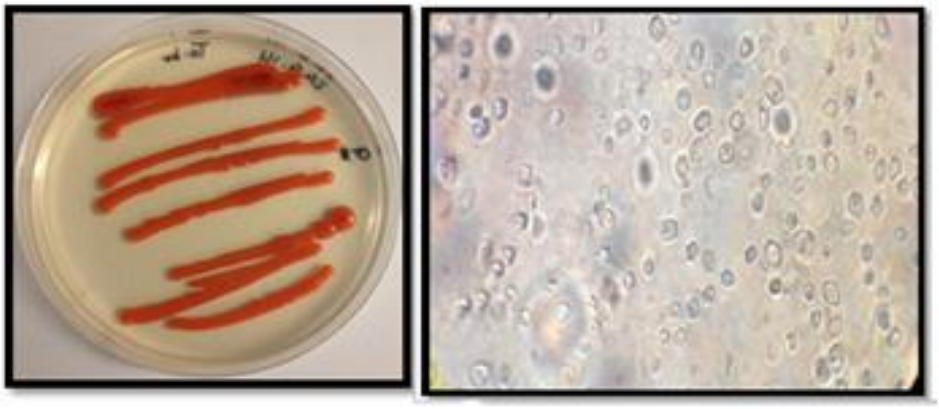
Yeast species were isolated using CIA.

1) Light microscope images.

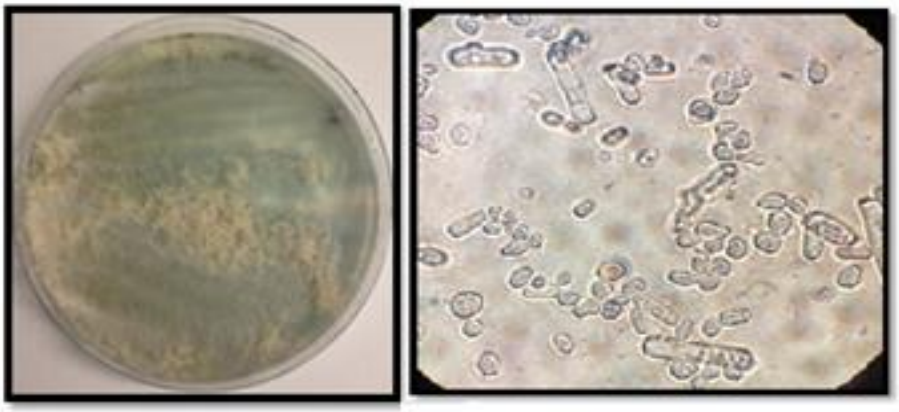
The isolates were examined under a light microscope after the yeast were identified.



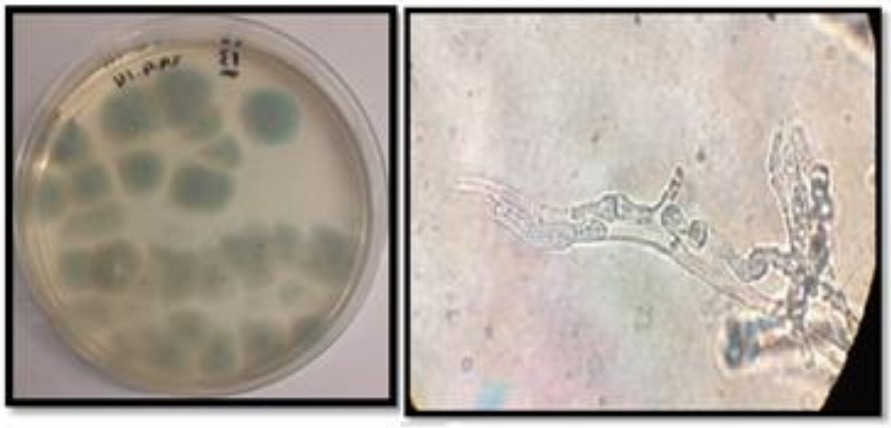
Neurospora tetrasperma.



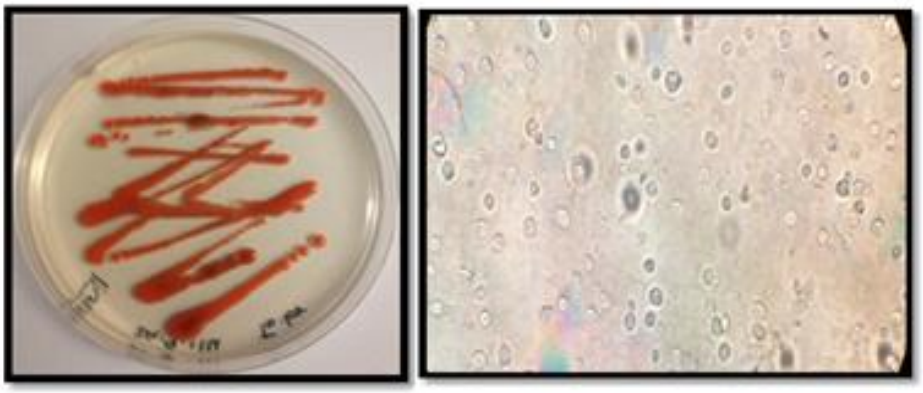
Rhodotorula mucilaginosa.



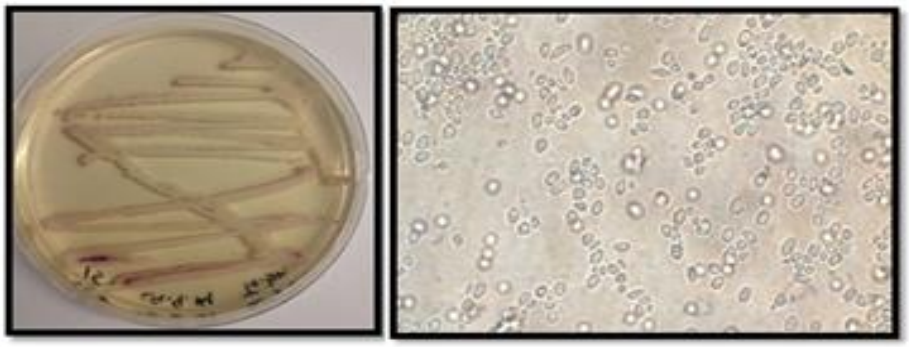
Sporidiobolales sp.



Chaetomium megalocarpum.



Rhodotorula.



Candida parapsilosis.

Figure 5.1: CIA plates showing a range of coloured mucoid isolates from toothbrushes (i.e. presumptive yeasts) and also showing examined *Candida* and other yeasts under the light microscope; the isolates were identified using 18SrRNA. The results are shown in Table 5.1.

Table 5.1: Yeasts and filamentous fungi isolated from various toothbrushes

Description	**Sequence Identity**	**Sequence ID**
Rhodotorula mucilaginosa	100%	KF953903.1
Neurospora tetrasperma	100%	JX136749.1
Sporidiobolales sp.	99%	EF060708.1
Chaetomium megalocarpum	98%	KC109743.1
Chaetomium globosum	98%	JQ964802.1
Rhodotorula	99%	KC206490.1
Candida parapsilosis	100%	KM113999.1

5.1.3. Discussion.

As with the other environmental samples, the isolates include both yeasts and filamentous fungi (*Rhodotorula mucilaginosa* and *Candida parapsilosis*) (Table 5.1). Again, the importance of these organisms lies not with their role as major pathogens, but by the fact that all can cause potentially life threatening problems in immunocompromised patients. Clearly,

only sterile, one-use tooth brushes should be used by such patients. Toothbrushes are the major oral hygiene device used to protect oral health due to microbial contamination and to prevent the possible transmission of infectious diseases. A new toothbrush becomes contaminated with pathogenic bacteria, viruses, and fungi within days of use, and these organisms potentially remain viable for various periods of times. Hygienic measures for the prevention of infection and re-infection are improving, where the contaminated toothbrushes remains a concern (Ankola *et al.*, 2009). Toothbrushes become contaminated with bacteria during daily use (Frazelle and Muro, 2012) and recontamination of the oral cavity may result from the retention of microorganisms on toothbrushes (Filho *et al.*, 2000). Public awareness is limited regarding contamination of toothbrushes with microorganisms as the result of regular use. This can be the cause of re-infection with pathogenic bacteria, or act as a reservoir for environmental micro-organisms (Karibasappa *et al.*, 2011). Nascimento *et al.*, (2012) showed that new toothbrushes may often possess a bacterial infection prior to use, since there is no requirement for pre-use sterilization. This observation was confirmed in our study, where all (100%) of new tested brushes were contaminated positive for bacterial growth, a finding which explains the high incidence of *Bacillus cereus* and *Candida albicans* on toothbrushes and oral swabs. Complete dryness of toothbrushes was found to prevent the multiplication of microbes. Exposed of toothbrushes to dust is also regarded as unsanitary (Verran and Leahy- Gilmartin, 1996; Karibasappa *et al*., 2011). Finally, it is noteworthy that *Candida* species can cause tooth cavities (Akpan and Morgan, 2002).

5.2. Scanning electron microscope studies of the surface of a used toothbrush.

Scanning electron microscope "SEM" studies was conducted on the surface of bristles of used toothbrushes. The brush head was firstly immersed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH7.3, for 60min and then brushes was rinsed in by using dehydrated in ethanol and critical point dried from liquid CO_2. A rotary saw was used to cut a segment of brush head with one row of bristles then mounted on aluminium stubs; gold sputter coated and examined using a JEOL JSM-5300lv scanning electron microscope at an accelerating voltage of 10–30 kV (Figure 5.2).

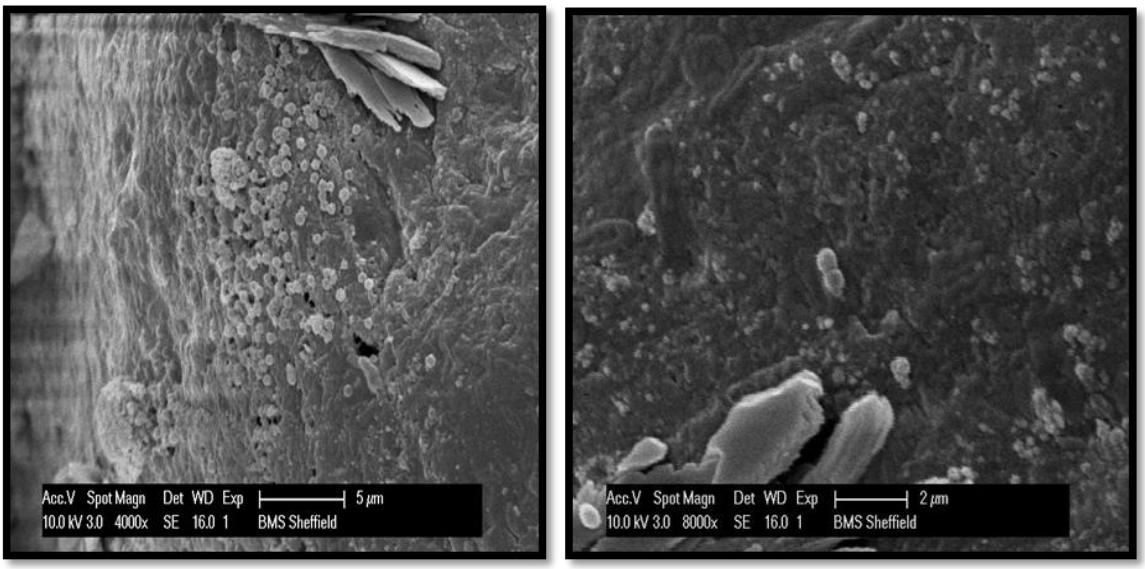


Figure 5.2: A microbial biofilm on an individual toothbrush bristle.

Scanning electron microscopy of biofilms of the working surfaces (i.e. bristles) of toothbrushes showed inorganic and microbial deposits. It is not obvious however, whether the cells seen are bacteria or yeasts or both. A microbial biofilm can be clearly seen (4000x bar represents 5 µm and 8000x bar represents 2 µm) on the surface of a single bristle for both scanning. It is not obvious whether the cells seen are bacteria or yeasts, although in relation to their size, they are probably coccoid bacteria. The left scanning showing the crystal seen in the top right hand corner is almost certainly a calcium salt (fluoride or phosphate).

5.3. Effect of toothpastes on the growth of yeasts.

The effectiveness of tooth brushes in cleaning teeth, and removing bacterial plaque, is largely dependent on their abrasive qualities. However, some tooth pastes contain antibacterial agents (which may also be general sterilants) such as hexachlorophene which also help remove plaque-causing bacteria and other oral microbes. Toothpastes generally contain a combination of fluoride and detergents, compounds which increase the efficacy of tooth-biofilm control (Davies 2008; Marsh 2010; Prasanth 2011). Triclosan for example, is a low-toxicity, non-ionic, chlorinated bisphenol that is compatible with toothpaste components, including fluoride and surfactants, and it enhances the inhibition of cyclooxygenase/lipoxygenase pathways and shows anti-inflammatory properties (Davies, 2008; Davies *et al.,* 2010). Chlorhexidine is generally considered to be the most effective antimicrobial agent, if used as mouthwash in dentistry (Jones, 1997; Twetman, 2004), due to its broad-spectrum action against Gram-positive and Gram-negative bacteria, viruses and yeasts. The effect is due to the dicationic nature of the chlorhexidine molecule, which provides a persistent antimicrobial effect on the surface of the tooth (Twetman 2004). However, attempts to formulate a toothpaste containing chlorhexidine have proved difficult, due to the inactivation of chlorhexidine by anionic ingredients (Davies, 2008; Davies *et al.,* 2010). Chemical agents may have side effects such as taste alteration, tongue and mucosa peeling or tooth staining. Novel phyto-therapeutic compounds have also been investigated (Verkaik, 2011; Pannuti, 2003); Parodontax for example, is a herb-based toothpaste containing sodium bicarbonate and several herb extracts such chamomile (anti-inflammatory), *Echinacea* (which stimulates immune response), salvia (decreases hemorrhage), myrrh (a natural antiseptic), and finally *Mentha piperita* (antiseptic, anti-inflammatory and antimicrobial) (Panuti, 2003). Toothpastes do not contain readily utilizable sugars, but do they provide carbon nutrients to support (along with dried sputum) the growth

and survival of yeasts on toothbrushes. The aim of the work reported in this section was to determine if yeast could be isolated from used tooth brushes.

5.3.1. Materials and Methods.

In order to determine the anti-yeast effect of toothpastes a toothpaste agar was used containing 100 Ml bacteriological agar and 4 g of a proprietary toothpaste in 8 laboratory bottles were shaken by hand and vortexed at low speed until complete mixing was achieved. The first agar was poured in half of petri dishes then left to cool; in the other half of petri dishes the mixed toothpaste agar was added and left to solidify. The medium in the plates was then streaked with either, *Rhodotorula* and *Candida parapsilosis*. The results, following incubation for 2- 4 days, are shown in (Figures 5.3; 5.4).

5.3.2. Results.

Figure 5.3: Commercial toothpastes used to prepare toothpaste agar plates.

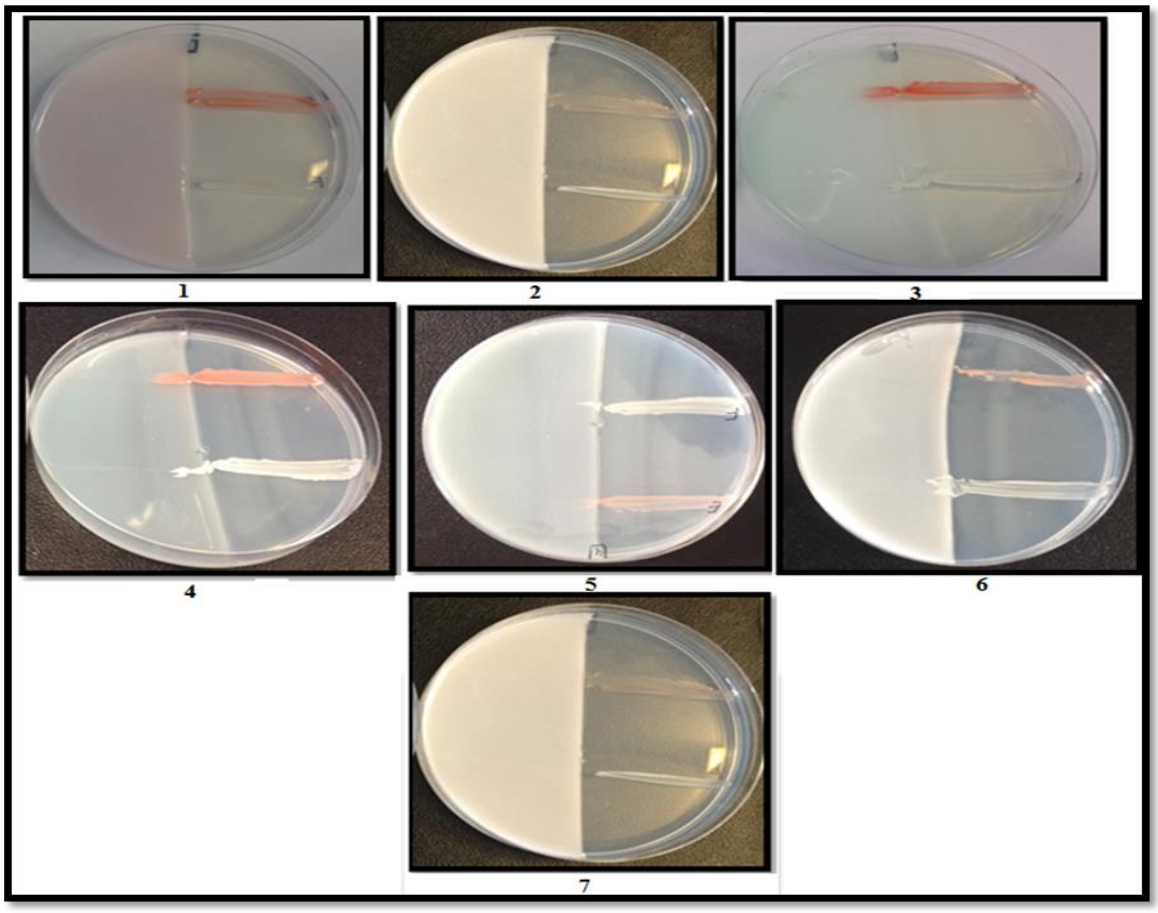


Figure 5.4: Inoculated commercial toothpastes agar plates by *Candida parapsilosis* (Colonies white) and *Rhodotorula* (Colonies orange/red).

5.3.3. Discussion.

As with the toothbrush samples, the isolates include yeasts and filamentous fungi (Table 5.1). Again, the importance of these organisms lies not with their role as major pathogens, but by the fact that all can cause potentially life threatening problems in immunocompromised patients (Akpan and Morgan, 2002). The results also highlight the need for public awareness about the health hazards resulting from these potentially troublesome microorganisms. Clearly, it is desirable that only sterile, one time-use tooth brushes should be used by immunocompromised. The results also show that toothpaste can act as a microbial nutrient source. Toothpastes do not contain readily utilizable sugars, but do they may provide

nutrients to support (including dried sputum and food particles) which can support the growth of yeasts and other microbes (Figure 5.1). In conclusion, the antibacterial effect of toothpastes was determined using seven types of commercial toothpastes and thus the results showed that the strains isolated from toothbrushes can grow on the bacteriological agar side, while, on the mixed toothpaste agar there was no yeast growth. A reasonable explanation for the observed inhibitory effect of toothpaste is based on it containing fluoride as an antimicrobial agent and also because of resultant pH effects. Results from other studies show that fluoride can affect yeast carbohydrate metabolism and it can inhibit essential enzymes (Marquis, 1995) and thus all fluoride toothpastes contain effective antimicrobial compounds (Figure 5.4).

CHAPTER 6

EFFECTIVENESS OF ANTIBACTERIAL CLOTHS IN INHIBITING THE GROWTH OF YEASTS.

The aim of this experiment was to determine the if a proprietary antibacterial cloth (e-cloth (EnivroProducts Ltd, East Bran, Furance Farm, Furance Lane, Lamberhurst, Kent,TN3 8LE) is useful in killing yeasts and other fungi when compared to a control, i.e., a normal non-antimicrobial cloth (Figure 6.1).

A B

Figure 6.1: A) The antibacterial e-cloth and **B)** non-antibacterial microfibre cleaning cloths.

6.1. Materials and Methods.

Two approaches were used:

Approach 1: Two types of silver impregnated cloths (antibacterial e-cloths “red colour” and a Non-antibacterial microfiber cleaning clothes “yellow colour”) were applied to environmental surfaces (desks, air conditioner, windows, medical equipment, laboratories). Pieces of cloth were moistened with sterile water and wiped firmly over the entire surfaces (Figure 6.1). The two types of cloths were then placed in 50 ml of Sabouraud Dextrose Broth (“SDB” for the selective cultivation of yeasts, molds and aciduric bacteria, final pH 5.6) which favors yeast growth and inhibits most bacteria) in sterile tubes, and vortexed for one minute and then left on the a shaker overnight. The SDB medium become turbid after 24 hours of inoculation due of the presence of fungi in the two types of cloths (Figure 6.2). Second approach: The same two types of cloths were placed on the surface of Sabouraud Maltose Agar ("SMA" for the selective isolation yeasts; final pH 5.6 (Chapman, 1952); incubation was at 25 °C for 3 to 5 days. After incubation the presence of yeast growth were noticed on the two types silver cloths (Figure 6.2).

6.2. Results.

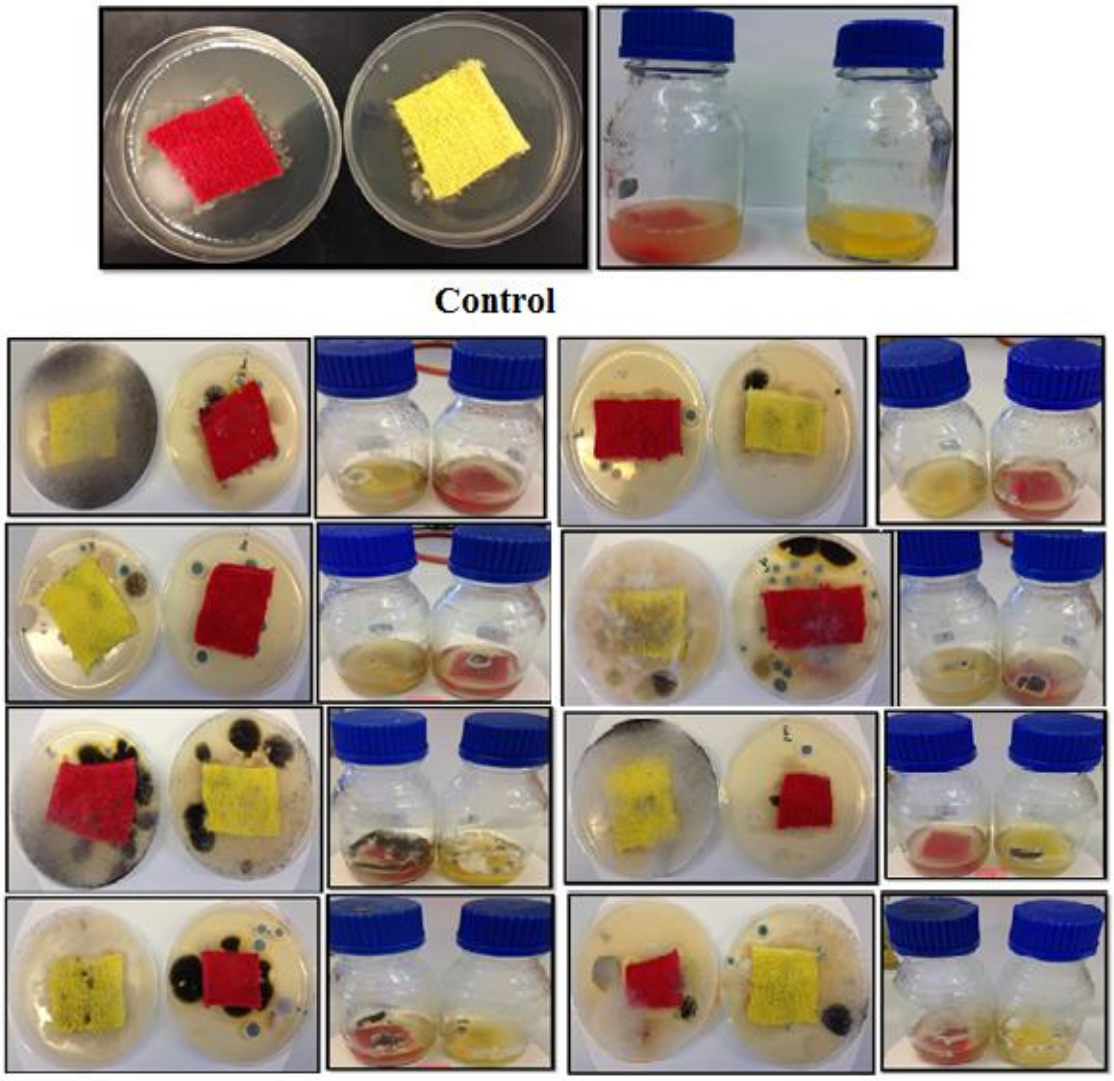
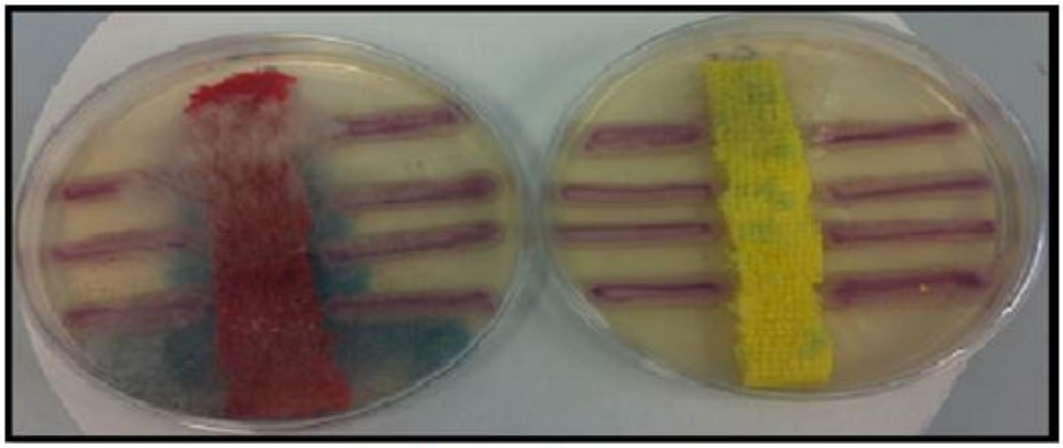
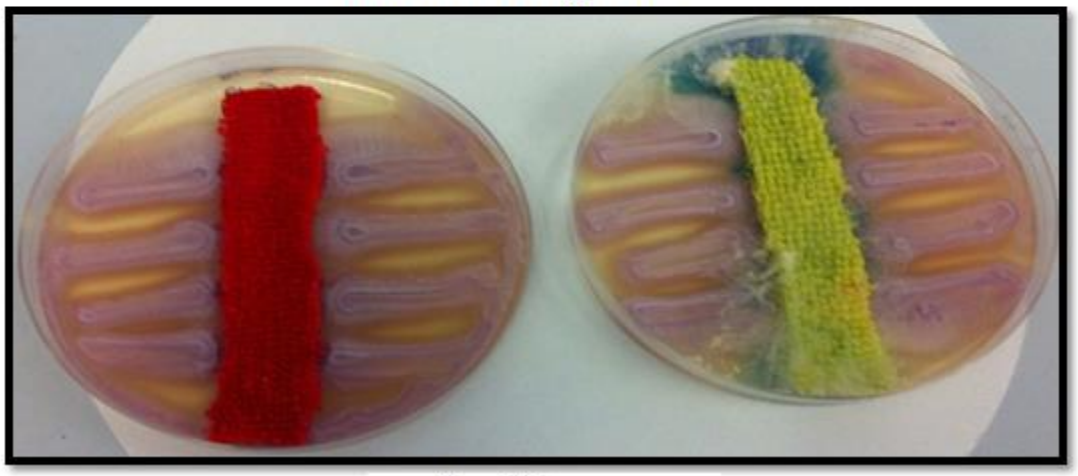


Figure 6.2: Growth of fungi around the antibacterial cloth "red colour" and control non-antibacterial cloth "yellow colour" cloths applied to various surfaces (desks, air conditioner, windows, medical equipment, laboratories) and placed on SMA medium.



Candida inconspicua



Candida rugosa

Figure 6.3: Yeast growth appeared adjacent to the antibacterial cloth (red colour) and control non-antibacterial cloth (yellow colour); i.e. the antibacterial cloth does not inhibit the growth of the yeasts studied. Note silver as bactericide might be expected to also kill yeasts.

6.3. Discussion.

When the silver impregnated cloth was wiped on various surfaces it became contaminated with dirt and dust. When this contaminated material was transferred to growth medium, fungi grew from it showing that the cloth is incapable of inhibiting surfaces contaminated with fungi, i.e. this proprietary silver-impregnated antibacterial cloth has no obvious anti-fungal activity. Similarly the antibacterial cloth did not prevent the growth of two yeasts when they were incubated adjacent to the material on a solid medium. It could of course be argued that

this result is to be expected as the cloth is marketed as an antibacterial and not anti-yeast or anti-yeast product. However, it contains silver which is a broad spectrum micro-biocide which inhibits the growth of fungi (Gupta and Chauhan, 2016), as well as bacteria. Presumably the concentration of silver needed to inhibit bacteria is lower than that needed to inhibit filamentous fungi and yeasts. As a result this antibacterial cloth would be of little use for controlling yeasts on surfaces in the hospital or household setting.

CHAPTER 7

STUDIES ON THE SURVIVAL OF YEASTS ON SMOOTH AND ROUGH UNGLAZED CERAMIC TILE UNDER AMBIENT CONDITIONS.

Ceramic tiles are widely used in the built environment and notably in hospitals. Such tiles provide an obvious survival environment for yeasts and other potentially pathogenic organisms. The aim of this work was to determine the survival (in the ambient environment) of a variety of yeast isolates on smooth (glazed) and rough (unglazed) tiles.

7.1. Materials and Methods.

The following types of tiles (Figure 7.1) were used: ceramic tiles (0.8 cm^2), smooth and rough were sterilized by autoclave 120^oC at 30 minutes. All experiments were set up and left in a biosafety hood. Swatches of tiles were lined up in rows next to, but not touching, each other. During the 3-day period of the study, the hood fan was left on; the temperature ranged from 22.9 to 24.5°C.

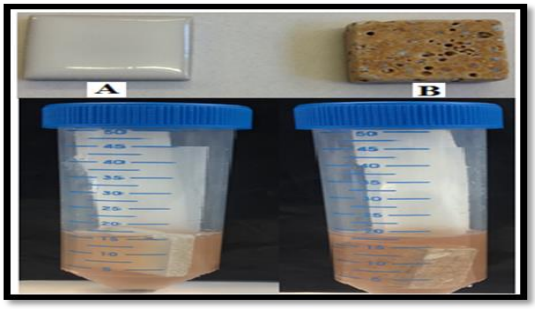
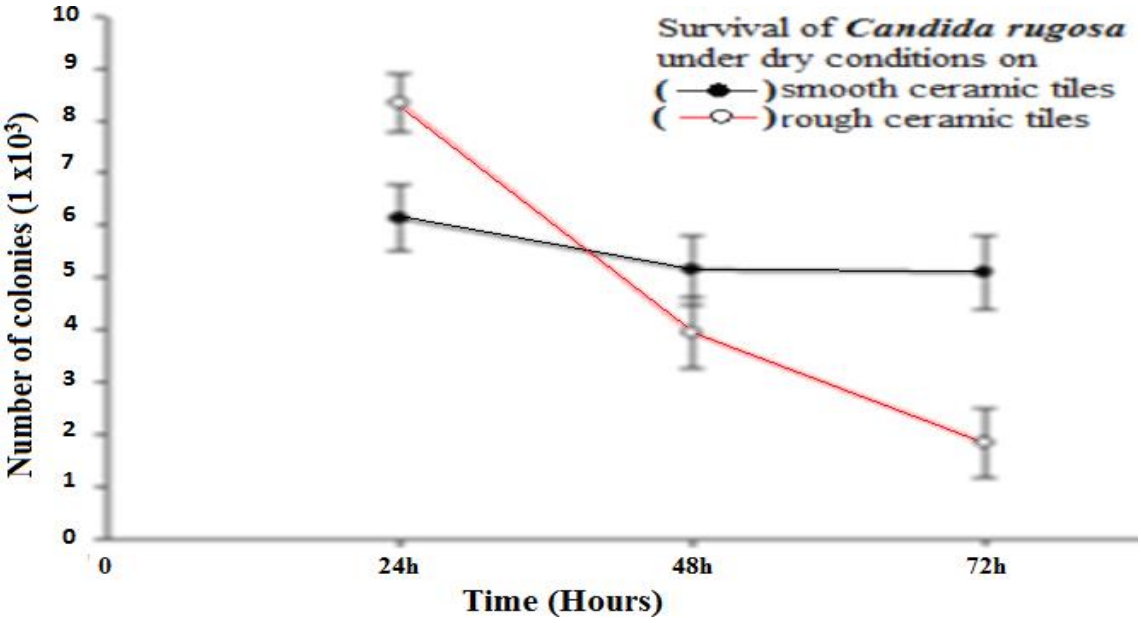


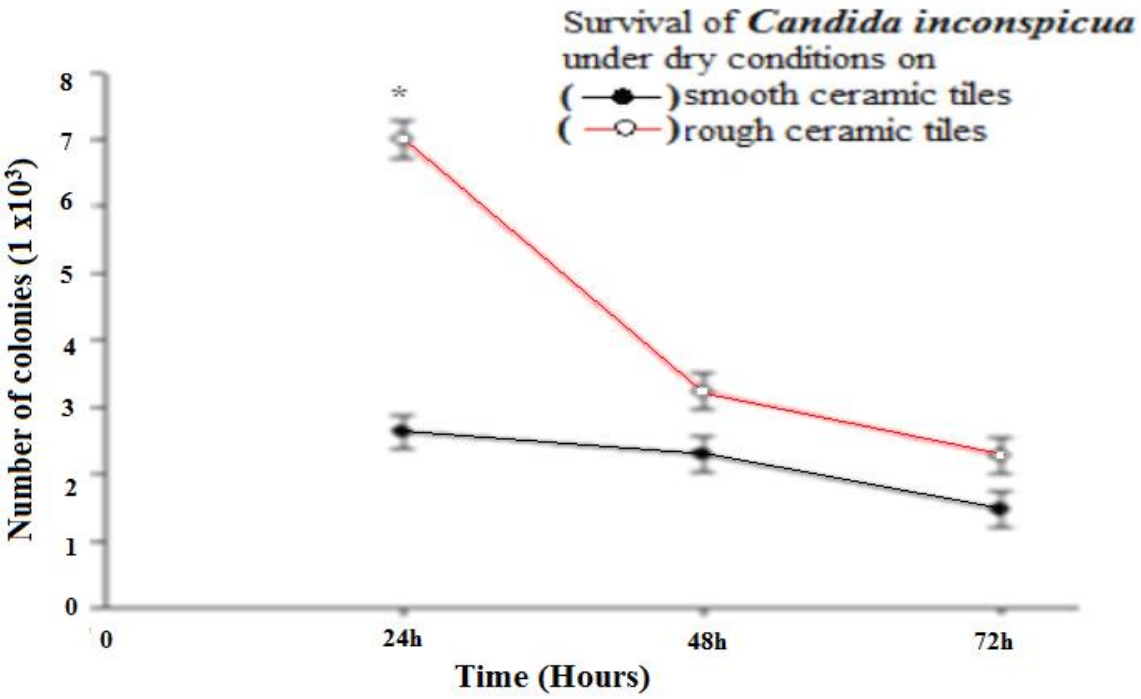
Figure 7.1: The two types of ceramic tiles were used, **A)** smooth, **B)** rough.

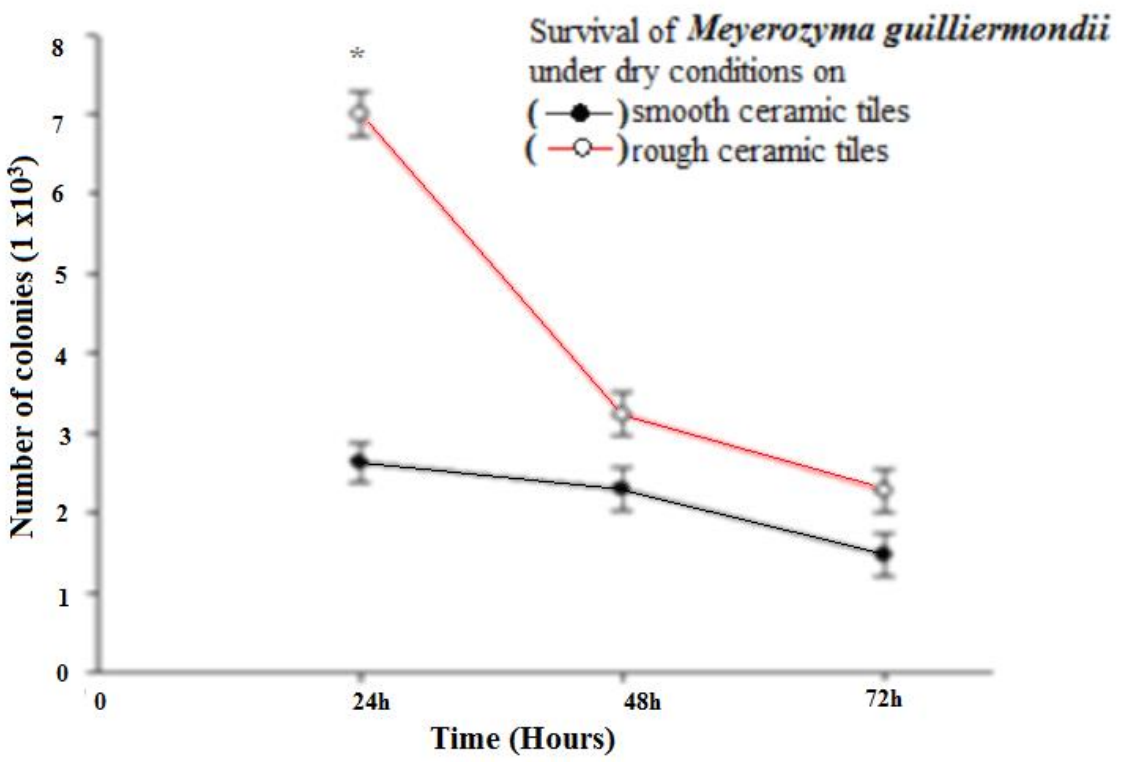
Using an Eppendorf pipette, tiles were inoculated with 10-ml aliquots of solutions with the desired concentration of the test yeast. Using an Eppendorf pipette, tiles were inoculated with 10-ml aliquots of solutions with the desired concentration of the test yeast. Immediately after inoculation and after the first 8 h, left in a biosafety hood then after 24 h, 3 tiles of each type were picked up with sterile forceps and transferred into sterile distilled water shaken and a

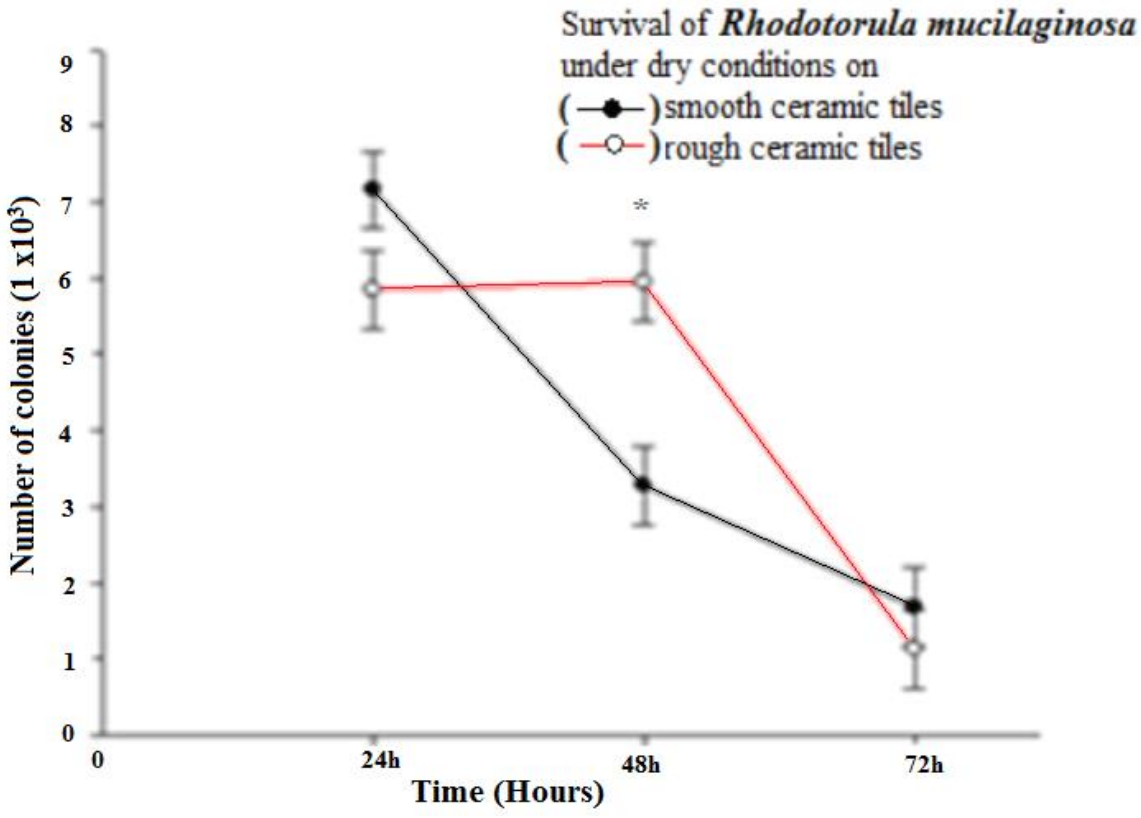
serial dilution was performed with incubation of the diluents on Sabouraud Dextrose Broth. The colonies which grew were then counted.

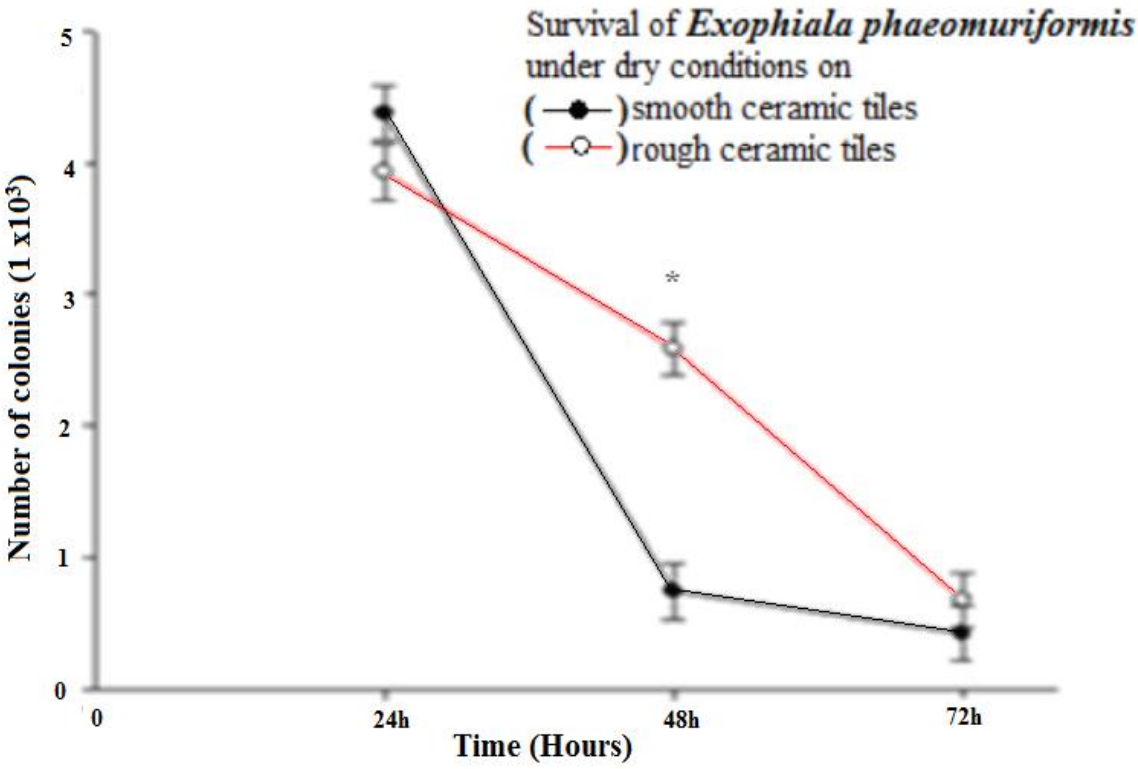
7.2. Results.

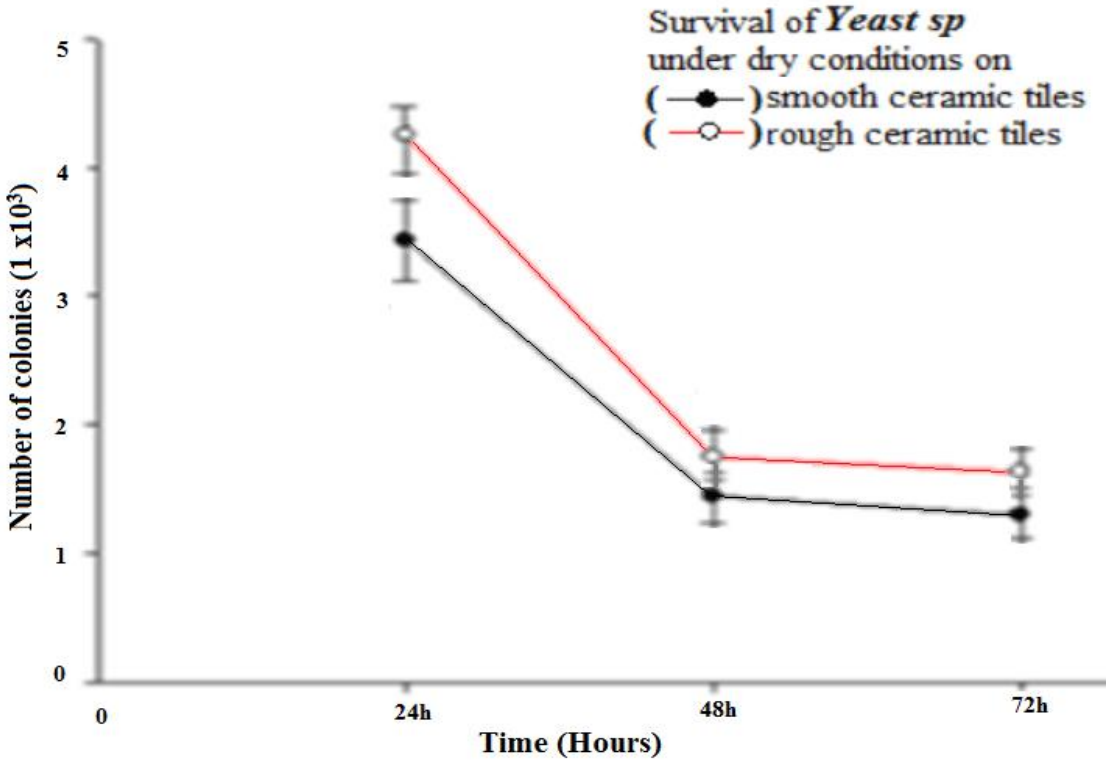


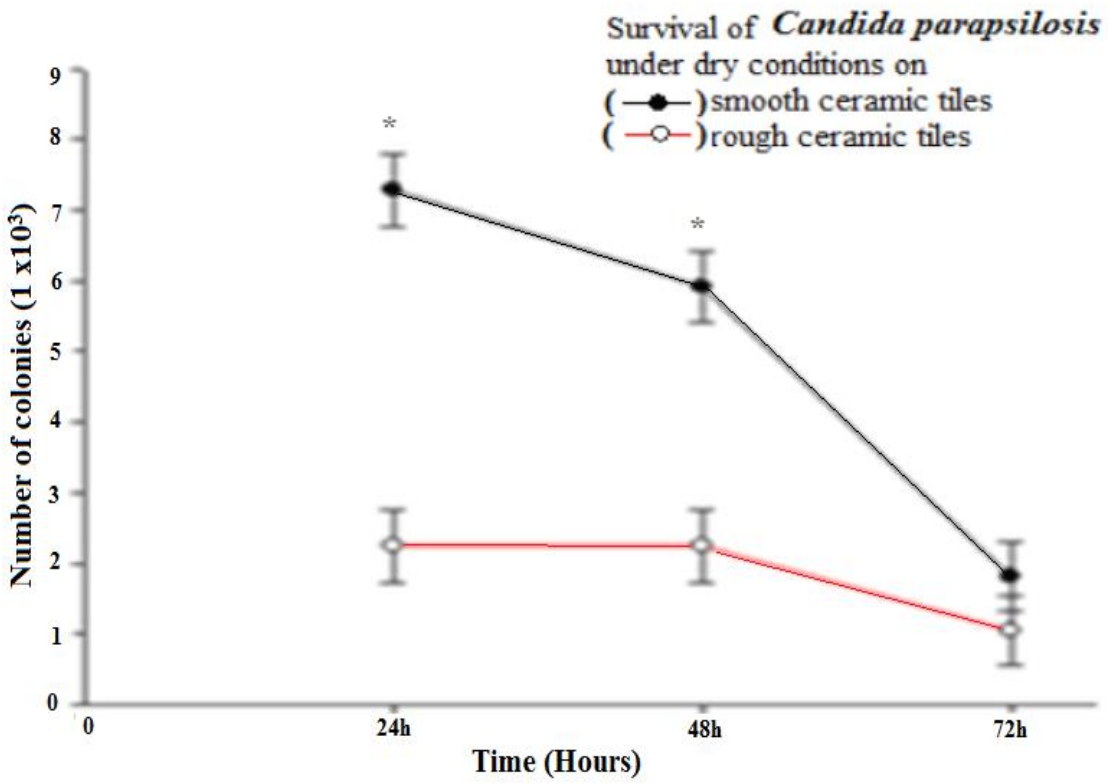


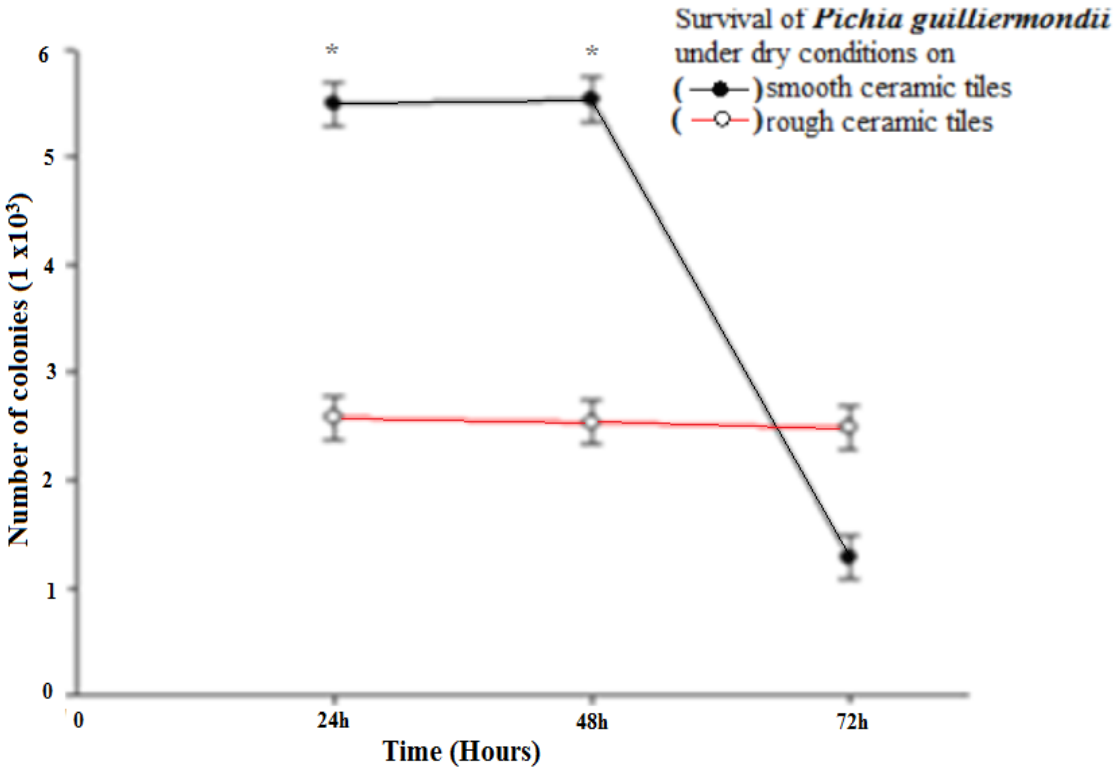

Survival of *Meyerozyma guilliermondii*
under dry conditions on
(—●—)smooth ceramic tiles
(—○—)rough ceramic tiles
*
Number of colonies (1 x10³)
0
1
2
3
4
5
6
7
8
0
24h
48h
72h
Time (Hours)

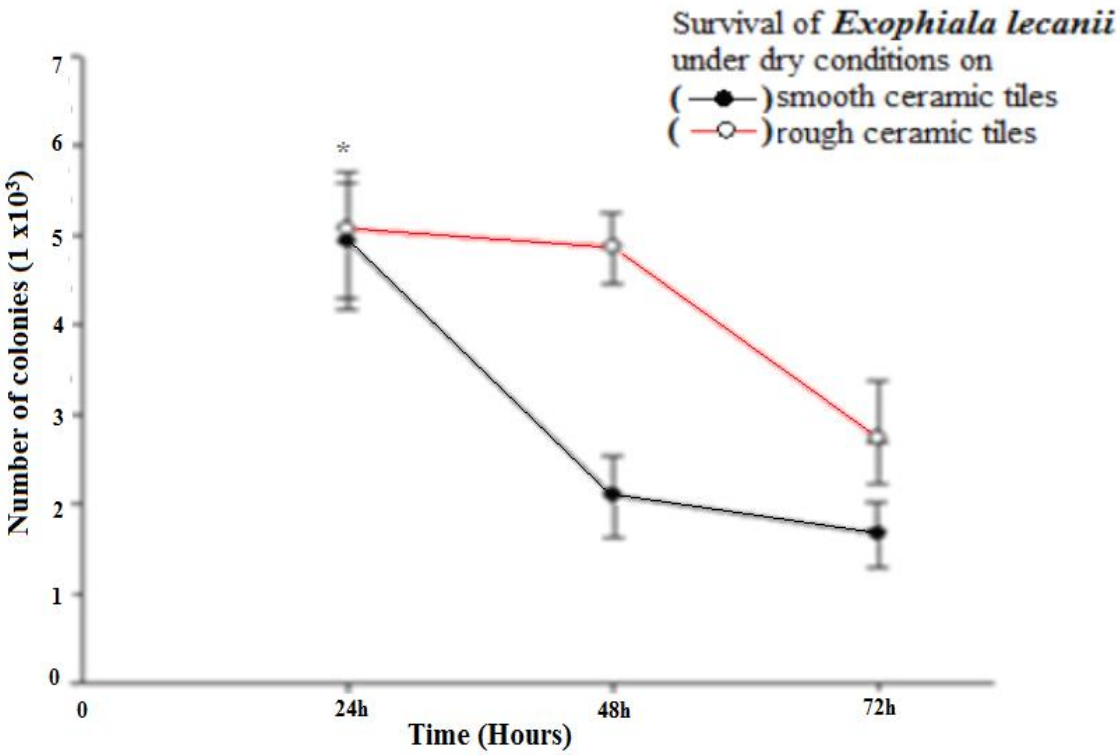

Survival of *Rhodotorula mucilaginosa*
under dry conditions on
(—●—)smooth ceramic tiles
(—○—)rough ceramic tiles
*
Number of colonies (1 x10³)
0
1
2
3
4
5
6
7
8
9
0
24h
48h
72h
Time (Hours)

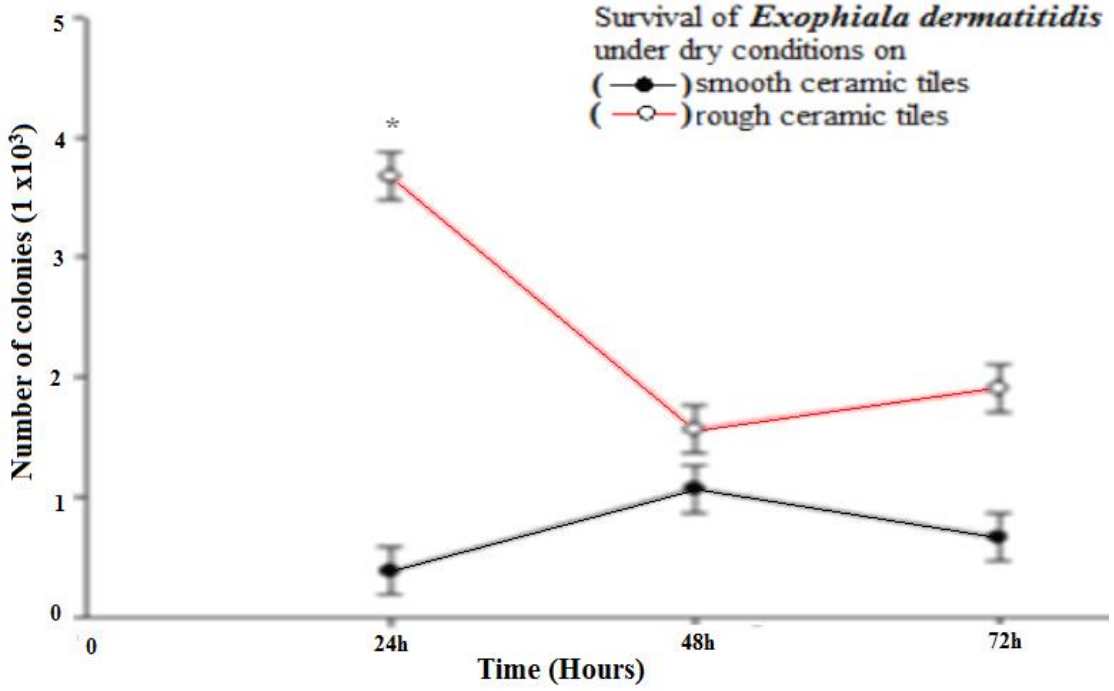

Survival of *Exophiala phaeomuriformis*
under dry conditions on
(—●—)smooth ceramic tiles
(—○—)rough ceramic tiles
Number of colonies (1 x10³)
5
4
3
2
1
0
*
0
24h
48h
72h
Time (Hours)


Survival of *Yeast sp*
under dry conditions on
(—●—)smooth ceramic tiles
(—○—)rough ceramic tiles
Number of colonies (1 x10³)
5
4
3
2
1
0
0
24h
48h
72h
Time (Hours)


Survival of Candida parapsilosis
under dry conditions on
(—●—)smooth ceramic tiles
(—○—)rough ceramic tiles
Number of colonies (1 x10³)
Time (Hours)
0
24h
48h
72h
*
*


Survival of Pichia guilliermondii
under dry conditions on
(—●—)smooth ceramic tiles
(—○—)rough ceramic tiles
Number of colonies (1 x10³)
Time (Hours)
0
24h
48h
72h
*
*


Survival of *Exophiala lecanii*
under dry conditions on
(—●—)smooth ceramic tiles
(—○—)rough ceramic tiles
Number of colonies (1 x10³)
Time (Hours)
0
24h
48h
72h
*


Survival of *Exophiala dermatitidis*
under dry conditions on
(—●—)smooth ceramic tiles
(—○—)rough ceramic tiles
Number of colonies (1 x10³)
Time (Hours)
0
24h
48h
72h
*

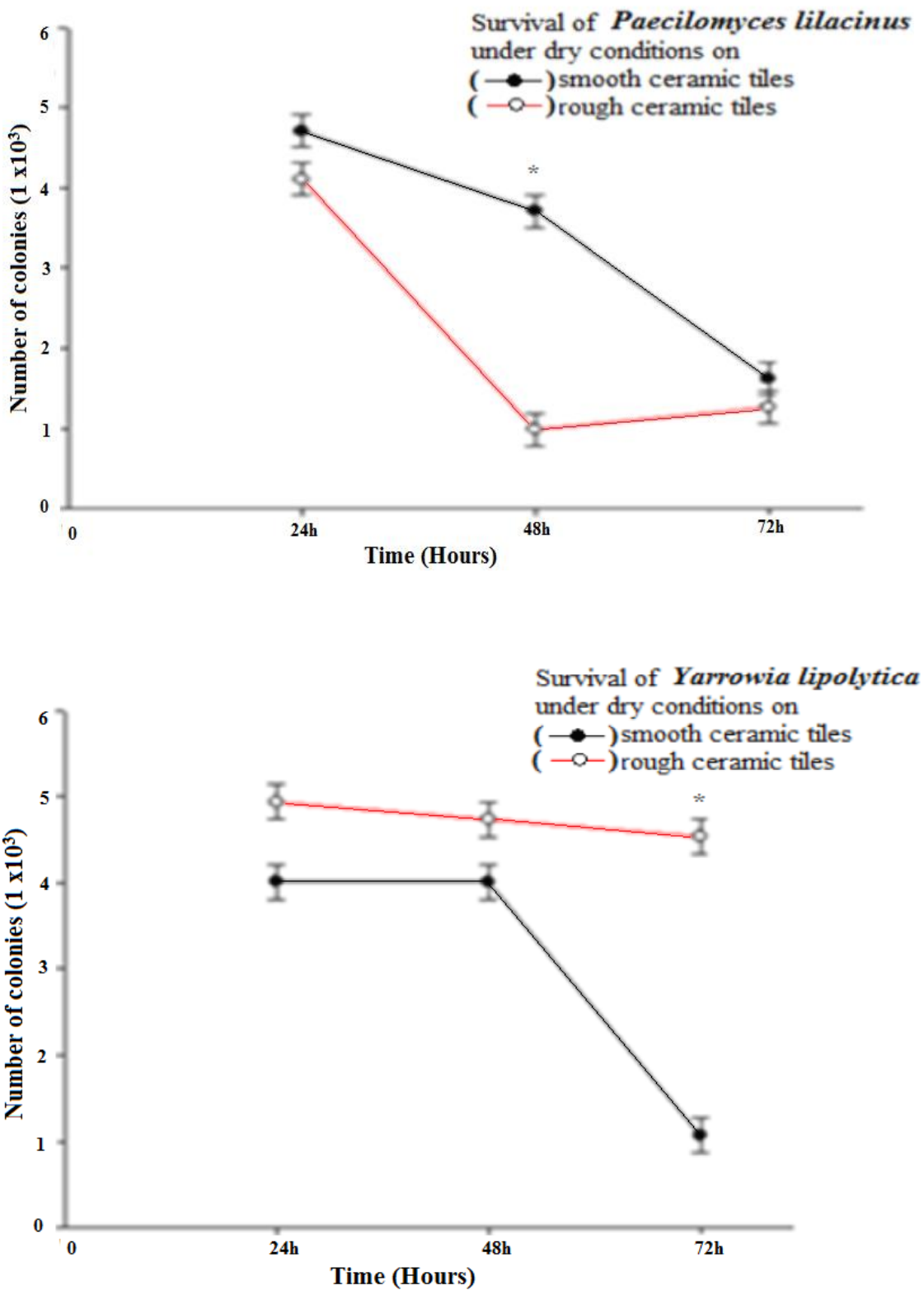


Figure 7.2: Survival of the yeasts on (—●—) smooth and (—○—) rough ceramic tiles under dry conditions.

Standard error (SE): ***** significant difference between tile types.

7.3. Discussion.

Yeast survival was tested on both smooth and rough ceramic tiles. These data show that many of the yeasts survived for at least a day; whereas, others survived for 3 days. These results show that ceramic tiles play a role as reservoirs or vectors for yeasts because those species tested generally remained viable on these surfaces for a number of days; the length of survival of the fungi on both smooth and rough ceramic tiles being related to the genus and species used. The most obvious feature of these results is that the yeasts studied generally appeared to survive for longer periods on smooth glazed tiles than on unglazed tiles. This finding is contrary to expectation, in that it would be expected that yeasts would be able to survive for longer in the moist cavities provided by the rough, unglazed tiles, especially since it would be expected that the smooth tiles would allow for rapid dehydration and direct exposure to sunlight- related UV. The unexpected findings obtained may result from an extraction anomaly, i.e. yeasts may survive for longer in the pores of rough tiles but these, unlike those present on smooth tiles, are not amenable to extraction, thereby giving the seemingly anomalous result that yeasts survive for longer on smooth tiles; of course the finding may reflect the true situation. We hope to develop experimental protocols to test the above in the near future. Yeasts such as *Candida rugosa* which is associated with nosocomial infections in patients, survived for 72 hours and often longer on surfaces routinely used in hospitals. This result agrees with the findings of Traoré *et al.,* (2002) who showed that *C. albicans* and *C. parapsilosis* remained viable for at least three to 14 days when dried on surfaces materials. Appropriate cleaning and disinfection of hospital tiles are obviously essential for optimal control of infections in hospitals. In contrast, yeast survival was tested on both smooth and rough ceramic tiles as explained previous. These results show that ceramic tiles play a role as reservoirs or vectors for fungi because those tested generally remained viable on these surfaces for many days. Strategies to reduce the rates of nosocomial

infection with these pathogens should conform to established guidelines, with an emphasis on thorough environmental cleaning and use of Environmental Protection Agency–approved detergent-disinfectants.

CHAPTER 8

INFLUENCE OF COPPER AND PLASTIC SURFACES ON THE SURVIVAL OF YEASTS IN RELATION TO THE HEALTH CARE ENVIRONMENT.

8.1. Introduction.

The transmission of microbes, including pathogens, between patients is largely via the hands of healthcare workers however, more generalized microbial contamination of the hospital environment can also occur (Noyce *et al.*, 2006) and inanimate surfaces have been shown to be the source for outbreaks of nosocomial infections. Most Gram-positive and Gram-negative bacteria survive for months on dry surfaces (Hota, 2004), whereas most viruses can persist on surfaces for only a few hours. In contrast, most yeast species can survive up to 4 months on dry surfaces an example being *Candida albicans*, which considered to be the most important of all nosocomial yeast pathogens. Factors which influence the survival of microbes on dry inanimate surfaces include temperature, humidity and exposure to sunlight (Kramer *et al.*, 2006). Metallic copper was registered at the U.S the first antimicrobial material and is now being re-evaluated as an antimicrobial material for use on touch surfaces, such as door handles, bathroom fixtures, and bed rails, notably in relation to attempts to reduce nosocomial infections (Grass *et al.*, 2011). The aim of the following experiments was to determine the survival of two yeasts (*Candida rugosae, Candida inconspicua*) on inanimate surfaces such as metallic copper surfaces and the plastics, materials which are often used in healthcare and hospitals environments. Three types of inanimate surfaces were used, namely copper pipes push-fit elbow 15mm, copper pipes compression stop end 15 mm and finally a plastic pipe connector 22mm " Wickes, Wickes Building Supplies Ltd, Northampton, NN5 7UG " (Figure 8.1).

Figure 8.1: Copper and plastic pipes used to evaluate yeast survival.

Weaver *et al*., (2010) tested the effectiveness of copper as an antifungal surface for use in air-conditioning systems as an alternative to aluminium. The results showed increased killing of fungi compared to aluminium. Copper also prevented the germination of spores present, thereby reducing the risk of spore release. Copper provided an antifungal surface and prevented subsequent germination of spores, as a result, it can provide a valuable alternative to aluminium for use in air-conditioning systems, notably in hospitals they concluded that fungi tolerate metal ions by entrapment within cell wall components, altering metal uptake, chelation or precipitation by secreted metabolites and intracellular binding by metallothioneins. Weismann *et al*. (2000) showed that *Candida albicans* is more resistant than is *Saccharomyces cerevisiae* to high levels of copper. They found that two genes were involved in this differential resistance, namely a metallothionein, CaCUP1, and a copper-trans-porting P-type ATPase, CaCRP1. Gene disruptions studies showed that a copper extrusion pump is responsible for the unusual resistance of *C. albicans* to copper, whereas the metallothionein is responsible for the residual copper resistance of the Cacrp1D mutant. In

acidic and anaerobic environments CaCRP1 function becomes essential for survival in the presence of even minute copper concentrations (Weismann *et al*. 2000).

By far the majority of work done on the survival of microbes on plumbing surface has been conducted using bacteria. As a result the following discussion is related to the survival of these organisms and not yeasts. Although there will obvious difference between the effects of, say cooper, in yeasts compared to bacteria, the information relating to the survival of bacteria on surfaces is of obvious interest to the findings discussed in this Thesis.

Bacteria are known to be inhibited by bacteria, the wild type strain of *P. aeruginosa* PAO1 for example, has a copper-inducible operon similar to *P. putida* KT2440 which was identified by sequence comparison (Quaranta *et al.,* 2007). In *P. aeruginosa* PAO1 the operon contains a *ptrA* sequence, and the arrangement of *cinRS* and *cinQA* on the chromosome is in opposite directions of those genes in *P. putida* KT2440. The sequences code for the proteins, cinS is a histidine sensor kinase, cinR a response regulator, cinA an azurine-like protein, cinQ a pre-Qo reductase. All genes could be induced and transcription activated by copper which indicated their function in copper homeostasis (Quaranta *et al.,* 2007). *Pseudomonas* spp. have different resistance mechanisms for copper. One of the first copper resistance systems described was the *copABCD* operon in *Pseudomonas syringae* pv *tomato*. The protein products were found to increase with cellular copper accumulation (Mellano and Cooksey, 1988; Cha and Cooksey, 1991). Homology was shown between the plasmid-borne copper-resistance genes *pcoABCDRSE* of *E. coli* and the *copABCD* and *copRS* determinants of *P. syringae* pv. tomato and *Xanthomonas campestris* (Rensing and Grass, 2003). Copper resistance mechanisms in *E. coli* have been well described by Rensing and Grass (2003). CopA is a P-type ATPase involved in removing Cu^{+} and Ag^{+} from the cytoplasm into the periplasm. It is co-regulated by CueR with CueO (cuprous oxidase) which oxidizes Cu^{+} to Cu^{++} and Ag^{+} to Ag^{++} in the periplasm. The CusCFBA proteins make up a multi-component

copper transport system which is regulated by cusRS. CusA is located in the inner membrane and serves as a proton-driven antiporter, CusC is an outer membrane factor, and CusB is a membrane fusion protein presumably interacting with CusA and CusC. CusF is a small periplasmic protein which binds single Cu^{+} and Ag^{+} ions and is necessary for maximum copper resistance. The genome of *Cronobacter sakazakii* strains implicated in neonatal meningitis cases were sequenced and *cusCFBA* genes identified which may be responsible in part for the bacterium's ability to invade brain microvascular endothelial cells (Kucerova *et al.,* 2010).

8.2. Copper resistance mechanisms in Gram-positive bacteria.

Gram-positive bacteria are different from Gram-negative bacteria in their cell wall structure. Gram-positives do not have a periplasmic space or an outer membrane but they have a thicker peptidoglycan layer. Copper ion-resistant strains of *Enterococcus faecium* used in this study were obtained from pigs in Denmark which were fed diets containing copper sulfate and antibiotics. One copper homeostasis mechanism described in *Enterococcus hirae* involves the *copYZAB* operon (Solioz and Vulpe, 1996; Wunderli-Ye and Solioz, 1999) which eliminates copper from the cytoplasm through membrane transporter proteins of the P-type ATPase family. The system has its own regulator, *copY,* which blocks transcription when bound to the *cop* promoter region on the chromosome. An increase in cellular copper results in dissociation of the CopY protein from the promoter and transcription of the *copYZAB* operon is initiated. *E. faecium* also maintains copper homeostasis through membrane efflux systems which are encoded by the chromosomal operon *copYZAB* which is similar to *copYZAB* in *E.hirae*. More recently transferable copper resistance genes (*tcrB* genes) have been identified as being part of the plasmid-borne *tcrYAZB* operon in *E. faecium* which is similar to the *copYZAB* operon and possibly mediates co-selection for resistance to macrolides and glycopeptides (Hasman and Aarestrup, 2002; Hasman *et al.,* 2006). Survival

rates of copper ion-resistant *E. faecium* strains on copper alloys revealed that differences in the membrane structure may also contribute to differences in survival times.

8.3. Mechanisms involved in contact-killing with copper alloys.

The antimicrobial properties of copper have been known for millennia and has been used in agriculture, water hygiene, healthcare environments, and food production, but without any investigations as to which mechanisms and environmental conditions were responsible for making copper so effective. It was not until the emergence of multiple antibiotic-resistant bacterial strains in the healthcare environment that the science community took a closer look at copper as an antimicrobial material. The copper content in copper alloys can vary from 60% to 99.9%, and initial studies have shown that the survival rates of *E. coli* O157 varied considerably with greatly on different alloys (Noyce *et al.*, 2006). The aforementioned copper resistance mechanisms in numerous bacteria are usually tested in copper ion-containing liquid and solid culture media which determine the minimal inhibitory copper concentration required for growth inhibition of a copper ion-sensitive or copper ion-resistant strain. Therefore, designing a study which would investigate the influence of genes involved in copper resistance seemed to be the next logical step in determining the mechanism of contact-killing on copper alloys. Certain environmental conditions have considerable influence on the survival of *P. aeruginosa* PAO1 as did the resistance genes themselves. For example, when cells were suspended in culture medium and placed as a droplet on a one-inch-square 99.9% copper alloy the survival times were twice those of cells where the aliquot was spread out over the entire surface. Thus, only by employing the "droplet method" differences in survival rates of the *cin* operon resistance genes mutant strains could be shown, which were tested at room temperature as well as at 4°C in order to demonstrate that these differences were indeed due to metabolic activity of the bacteria. Since these results indicated a gene-mediated response to survival on copper alloys in *P. aeruginosa* PAO1 the next part of the study was

designed with *E. coli and E. faecium* strains which had increased resistance to copper ions due to additional plasmid-borne copper resistance genes. In these experiments it was noted that the greatest differences in survival rates were related to moisture content in the sample, the type of medium the bacteria were suspended in, and whether it was a Gram negative or a Gram-positive bacterium. Since copper ion toxicity had been reported previously as a possible driving force in the contact-killing of *E. coli* on copper alloys (Espirito Santo *et al.*, 2008) an experiment was designed to block copper ions released from the copper surface by applying a corrosion inhibitor which would consequently prevent copper ions from entering the bacteria. This was correlated to electrochemical measurements and calculated concentrations of copper released from the surface, and an inverse relationship was shown between the copper ion concentration released from the surface and survival rates of copper ion-resistant *E. coli*. Subsequently it has been shown that bacterial cells very quickly take up copper ions when exposed to copper surfaces which results in rapid cell death presumably related to increased oxidative stress (Grass *et al*., 2010; Espirito Santo *et al.*, 2011).

8.4. The fate of copper ion-resistant bacteria.

Exposure to antimicrobial copper alloys under certain environmental conditions leads to rapid killing of copper ion-resistant bacteria and presumably does neither allow for increasing copper resistance due to the exposure, nor for proliferation of copper resistance via horizontal gene transfer. These findings are equally important for antibiotic-resistant bacteria. Copper surfaces are now being tested in hospitals where their efficacy is compared to stainless steel touch surfaces for long term observation, isolation, and characterization of surviving microbes. However, bacteria will continue to acquire resistance and will continue to proliferate in natural environments as a result of exposure to pollution of anthropogenic origin. Here copper resistance can be quite useful, such as in bioremediation of copper mining wastelands. This possibility is evaluated in the last part of the study since new and re-

discovered uses for antimicrobial copper alloys may result in increased copper mining and production.

8.5. Materials and Methods.

Copper pipes were sterilized by autoclaving and plastic pipes were sterilized using a 10% ethanol spray and yeast survival on each inoculated pipe was compared with uninoculated control pipes; the yeast suspensions was obtained from the colonies and transferred to sterile saline- equal to McFarland 0.5 turbidity. Pipes were inoculated with a yeast suspension (10mls), left to dry for 30 mins. and then left for a further 15 days under sterile conditions. The numbers of viable organisms in the suspension were determined by serial dilution and plating on CIA; incubation was at 25 °C for 3 to 5 days (Figure 8.2).

8.6. Results.

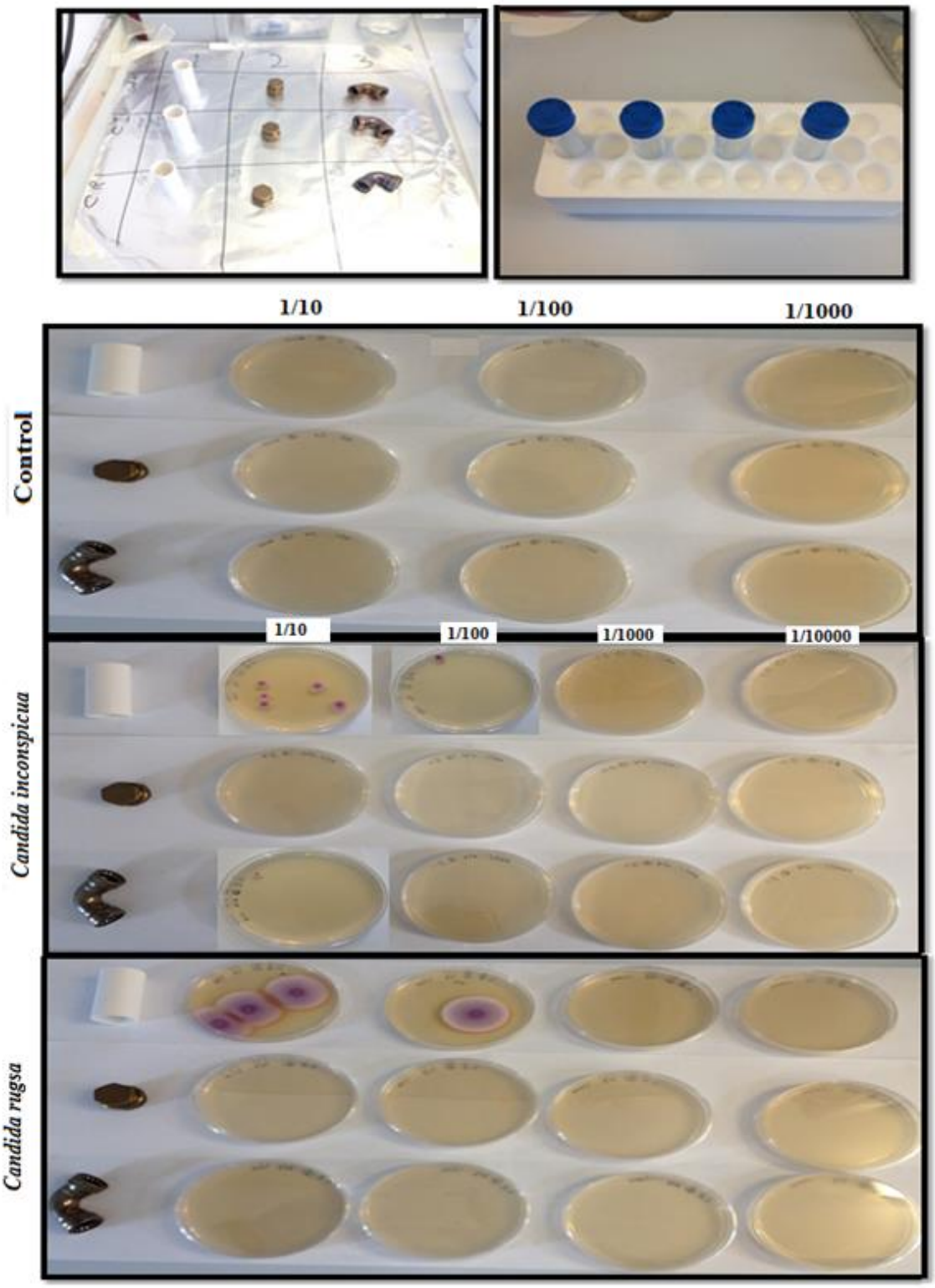


Figure 8.2: Non-survival of *Candida rugosa, Candida inconspicua* inoculated onto cooper and plastic pipes.

8.7. Discussion.

As shown by Figure 8.2, complete killing was achieved on copper pipe for both yeasts within the 15 day exposure period, while the yeasts survived on plastic piping over this time period. The survival rate of yeast on plastic pipe and growth was obtained after 20 days. In view of the above results, the use of copper materials is likely to reduce microbial contamination in hospitals, when present in small amounts, copper is a microbial nutrient, but is high concentrations it is highly toxic and leads to rapid DNA fragmentation and cell death and as a result it is a potent biocide (Warnes *et al.*, 2010). The replacement of copper with other materials should be avoided in order to minimize the risk of hospital-acquired infection and the transfer of these between health care workers and patients.

CHAPTER 9

9.1. FINAL DISCUSSION.

It is clear from the work provided in this Thesis that yeasts and other fungi commonly contaminate everyday objects and surfaces. While this reality does not present a problem to healthy individuals in their normal life it is an obvious issue for hospitals and other healthcare environments. Emphasis has been placed throughout this study on the problems posed to immunocompromised patients by infection by ostensibly non-pathogenic microbes. In fact, it seems that any microorganism can act as pathogen to patients with a weakened immune system and this fact, as we have seen, is certainly true for yeasts and filamentous fungi.

As a result of this reality, it is obviously necessary for all surfaces within healthcare environments to be kept clean in order to reduce the environmental load as far as is possible. This can be achieved by overall cleanliness and the use of biocides. Part of the following Discussion will now be devoted to a consideration of this important issue. Rather than give individual references to the points made here, the reader is referred to the following general reviews on the subject: Dancer, (2004, 2008, 20011), Al-Hamad and Maxwell (2008) and Patel *et al.,* 2007.

Cleaning is the primary defence against the threat of 'super bugs', influenza viruses, yeasts and other fungi and more recently *E.coli, C.difficile*, Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Enterococcus* (VRE). The importance of cleaning, disinfecting and sterilizing in healthcare settings is widely accepted and its implementation is usually strictly enforced, in community settings such as schools, offices, retail, hotels, public transport etc; the importance of remains important, but is less emphasised.

Bleach is the general standby for all cleaning and disinfecting purposes, but hydrogen peroxide is a promising alternative and can be used as an effective disinfectant for both

critical and non-critical surfaces. It exhibits rapid and effective broad spectrum antimicrobial activities against spores, mycobacteria, viruses, fungi and bacteria. Steam vapour systems and microfibers are also effective disinfection approaches for us in routine cleaning. The contact time between a disinfectant and the object being treated is extremely important although contact time is not strictly enforced within the cleaning industry. Contact times can vary however, dependent on a number of factors, notably microbial loading.

There are a number of modes of transmission of pathogens including:

Droplet contact transmission: Many diseases are transferred by infected droplets contacting surfaces of the eye, nose or mouth. Such droplets containing microbes can be produced when an infected person coughs, sneezes or talks; examples include SARS, the common cold, Legionnaires', disease and MRSA.

Airborne Transmission: Droplet nuclei (residue from evaporated droplets), or dust particles which contain microorganisms can remain suspended in the air for long time periods of time. Such organisms enter the upper and lower respiratory tracts and include diseases such as chickenpox, measles, tuberculosis and aspergillosis.

Faecal-oral transmission: Microbes enter the body via the consumption of contaminated food and water and then multiply inside the digestive system; they are also shed from the body via faeces. Poor hygiene and sanitation allows these microbes to contaminate food, water or environmental surfaces. Diseases contracted via this route include *E.coli*, *Cryptosporidium, C. difficile, H. pylori, Candida* infections rotavirus and hepatitis A virus (HAV).

Direct contact transmission: Requires physical contact between an infected person and a susceptible person, thereby allowing for the physical transfer of microbes. This may occur through kissing, sexual contact or merely close contact living between individuals of the

same household. Direct contact transmission diseases include *Acinetobacter*, *E.coli*, SARS, the common cold, ringworm and other yeast infections, scarlet fever, norovirus, foot and mouth disease, *H.pylori*, MRSA, H1N1 virus and hepatitis A virus (HAV).

Indirect contact transmission: Occurs when a susceptible person comes into direct physical contact with a contaminated surface via hands, and then goes on to touches their face, eyes or mouth, allowing the pathogen to enter the body, thereby resulting in infection. Diseases transmitted via this route include: norovirus, *C. difficile,* MRSA, SARS, *E.coli, Cryptosporidium,* ringworm and other yeast infections, scarlet fever, hand foot and mouth disease, *rotavirus,* hepatitis A virus (HAV), influenza, the common cold, *H. pylori* and tuberculosis. Such organisms can survive on surfaces for long periods and it is therefore important to eliminate surface contamination through cleaning and disinfection.

Use of detergents, disinfectant or sterilizers.

The use of detergents, disinfectants and sterilizers varies and is based upon their cleaning function and efficiency, environmental impact, cost, and toxicity.

Detergents.

These are low cost and effective on noncritical surfaces which make a minimal contribution to infection transmission and cause minimal damage to environmental surfaces and do not present disposal or occupational health exposure issues. They usually do not exhibit persistent antimicrobial activity and often only remove microbes rather than killing them, and their residues can serve as a nutrient source for surviving microbes. Detergents require rubbing and scrubbing to achieve full effectiveness, but are good at removing fool spills, chemicals and other commercial wastes. They are however, not suitable for cleaning potentially infective material such as blood or other body fluids, and are generally not effective against bacterial spores, notably this of *C. difficile.*

Sterilizing agents.

These are effective on all environmental surfaces, but are mainly used on critical surfaces. They can cause significant damage and degradation of environmental surfaces and are suitable for use in industrial, laboratory and hospital environments, although they are not generally used for day-to-day cleaning. Unfortunately they are often expensive and highly toxic and can lead to health risks. On the plus side, they are very effective in killing pathogens including spores of *C. difficile.* Examples include heat, chemicals, irradiation, filtration and high pressure. Such agents are very effective in cleaning potentially infective material such as blood and other body fluids.

Some guidelines for decontaminating environmental surfaces.

Noncritical Surfaces.

Routine detergent-based cleaning is sufficient to prevent disease transmission from non-critical environmental, i.e. surfaces of furniture and floors except where there is continual floor contact, including in crèches and nurseries. Low-level disinfection is recommended in these situations. All soil should be removed by wiping or scrubbing prior to the use of a cleaning agent. Dry conditions allow for the survival of gram-positive cocci (e.g., coagulase-negative *Staphylococcus* spp.), *Acinetobacter*, CA-MRSA, *Aspergillus* and other fungi in dust and on surfaces. Damp-dusting with cloths moistened with detergent is recommended for most non-critical surfaces and since moist environments favour the growth and persistence of bacteria fungi, after cleaning all surfaces need to be dried thoroughly. For health care settings in general, all radiators (including between panels) should be clean and dust and spillage free, as should fans, air conditioning units, switches, sockets or data/computer points. Doors, including edges should be clean, especially high touch areas such as handles and push plates. Ventilation grilles, extractors and inlets should be dusted weekly and fully washed each year.

Unused taps or shower heads should be run at least once a week for 10 minutes in order to control Legionnaires' disease. However, unless they are visibly soiled, walls and ceilings need only be washed every 6 months using hot water and detergent.

The contact time of a disinfectant is the length of time the surface needs to remain wet with the agent to allow for killing of particularly resistant microbes; strict following of disinfectant contact time recommendations must therefore be followed.

The following factors affect disinfectant choice:

- Compliance with chemical safety regulations.
- Material compatibility- some disinfectants corrode or discolour surfaces.
- Antimicrobial activity- dose the product posses a wide antimicrobial spectrum?
- Contact times- what is the minimum exposure time needed to reduce the microbial load by around 99.9%.
- Storage and shelf life- Is the agent stable?
- Disposal-Are there issues present regarding disposal and is the agent an environmental toxicant?
- Does the disinfectant work in the presence of organic matter?
- Does temperature and pH affect the disinfectant's effectiveness?
- Finally, is cost prohibitive?

Disinfectants used Routine in Cleaning.

The following are the most commonly used, EPA-registered disinfectants:

- Ethyl or isopropyl alcohol (70-90%).
- Sodium hypochlorite, i.e. bleach (5.25-6.15% household bleach diluted 1:500 provides >100 ppm available chlorine).
- Phenolic and Iodophor germicides.
- Quaternary ammonium germicidal detergents.

- Hydrogen peroxide (3-7.5%).

Environmentally safe disinfectants.

So-called ‘environmentally safe’ disinfectants include baking soda, vinegar, borax, liquid detergent, eucalyptus oil, grapefruit seed extract, grain alcohol, tea tree oil. Most environmentally safe disinfectants do not kill *S.typhi* and *E.coli*, with the possible exception of undiluted vinegar and eucalyptus oil. Eucalyptus oil has broad spectrum antimicrobial activity against bacteria including mycobacterium and MSRA, viruses and fungi (including *Candida*).

Antibacterial coating of surfaces.

Antimicrobial coatings are available for linen (curtains); furniture (lockers and bedside tables); office equipment (computers and printers); hand-touch sites (door handles and taps) and general surfaces (floors, walls and doors). Anything that can be impregnated or coated with a microbicidal paint or chemical can be marketed as ‘antibacterial’. Bioactive surfaces or coatings generally contain heavy metals, notably copper, or antiseptics and biocides. Electrostatic and inhibitory surfaces can also be used to prevent microbe-adhesion. Nano-silver particles combined with titanium dioxide are the most recent and potentially useful of these which from an invisible protective nano-coating applicable for use on a range of surfaces under low temperatures.

The majority of *Candida* infections are probably the result of endogenous sources arising from patients and molecular typing studies of yeast isolated from patients, from the hands of health care workers, and from the general environment shows that fomites may also play a role in the spread of *Candida albicans, Candida glabrata,* and *Candida parapsilosis* among patients who have undergone bone marrow transplantation (Panakcal, 2013). The experimental inoculation of dry surfaces shows that *C. albicans* and *C. parapsilosis* can survive for 3 and 14 days, respectively (Taore *et. al.,* 2002). *Aspergillus* and *Zygomycetes*

species are causes of nosocomial skin infection that originate from contaminated fomites and infections have been associated with the use of arm boards or bandages by patients who have intravascular catheters, in addition to the use of elasticized surgical bandages, hospital construction activity, and postoperative wounds (Beck-Sague et al., 1993).

9.2. CONCLUSION.

In conclusion the work described in this Thesis has shown that three species of *Candida* were isolated from bovine dung but not a mineral soil; two for these were shown to hydrolyze urea, oxidize ammonium to nitrate, solubilise elemental sulfur and solubilise a source of insoluble phosphate. A wide range of yeast and filamentous fungi were isolated from computer keyboards, sinks, used tooth brushes and vacuum cleaner dust. Yeasts were also shown to be emitted by hand dryers, the use of which is becoming increasingly popular. It is suggested paper towels be used in preference to these machines in health care settings, as hand dryers can spread potential pathogens into the air. Studies were also made on the ability of yeasts to survive on smooth and rough tiles similar to those found in homes and hospitals. Paradoxically, yeasts were shown to survive longer on smooth, compared to rough tiles and on plastic plumbing fittings than on copper fittings; in fact copper fittings appear ideal as they were seen to dramatically reduce the survival of potentially pathogenic yeasts and, as a result, should be used in preference to plastic piping for use in hospitals and other healthcare settings. The results presented here show that yeasts are important nosocomial pathogens in relation to life threatening diseases in immunocompromised patients and there presence and survival in healthcare settings is an important area of research which deserves much more extensive investigation.

9.3. SUGGESTIONS FOR FURTHER STUDIES.

The work presented in this Thesis has provided a "shotgun approach" to the isolation of yeasts from the environment, notably the built environment in relation to health care settings.

1) Any of the areas of study touched upon here could be investigated in greater depth in order to better appreciate the distribution of yeasts in the environment and their survival strategies they employ.

2) It would be desirable to determine if yeast and other fungi can perform the mineral cycling-transformations studied here *in vivo*, since microorganisms are likely to be able to perform such transformations in nutrient rich media, but not in the nutrient-poor conditions which are generally found in the environment.

3) Three particularly important areas of research covered in this study need to be re-visited, namely the survival of potentially pathogenic yeasts on tile, different types of piping used in plumbing and finally the role of hand dryers in distributing yeasts and other microbes around the indoor medical environment.

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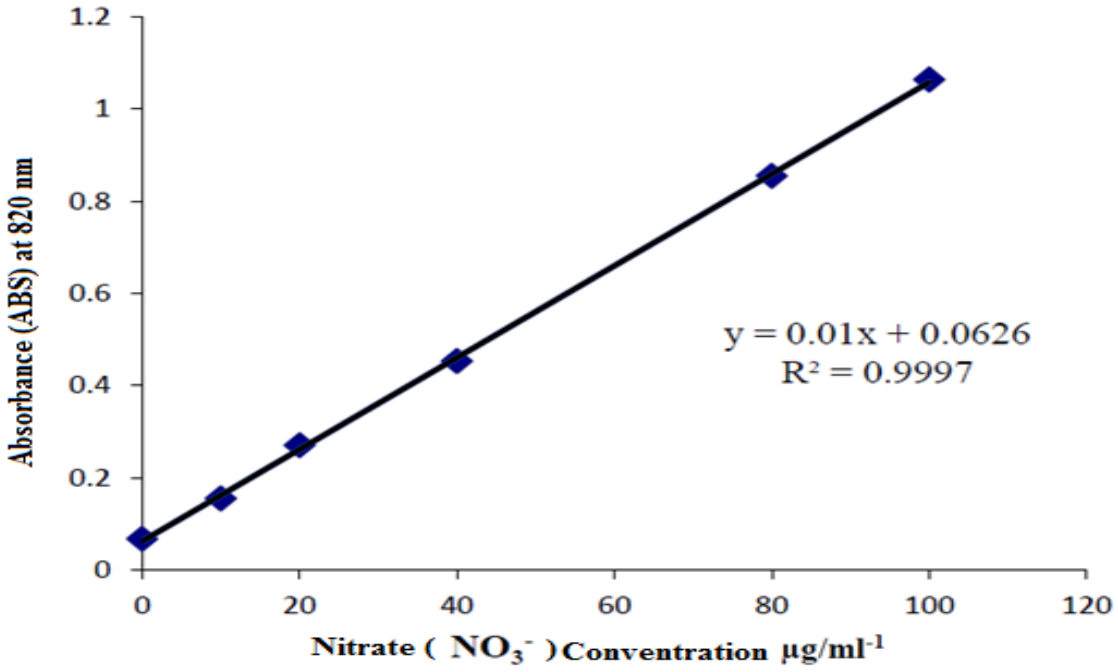
APPENDIX: A

(1) Standard curves preparation

- Standard curves for Nitrate

1. 1.37 g of sodium nitrate ($NaNO_3$) was dissolved in 100 ml of distilled water dH_2O to obtain 1µg of nitrate per µl (mixture 1).
2. 10 ml of mixture 1 was diluted into 90 ml of distilled water dH_2O for 10 times (mixture 2).
3. Mixture 2 was diluted with dH_2O to produce solution 0, 10, 20, 40, 60, 80 and 100 µg.

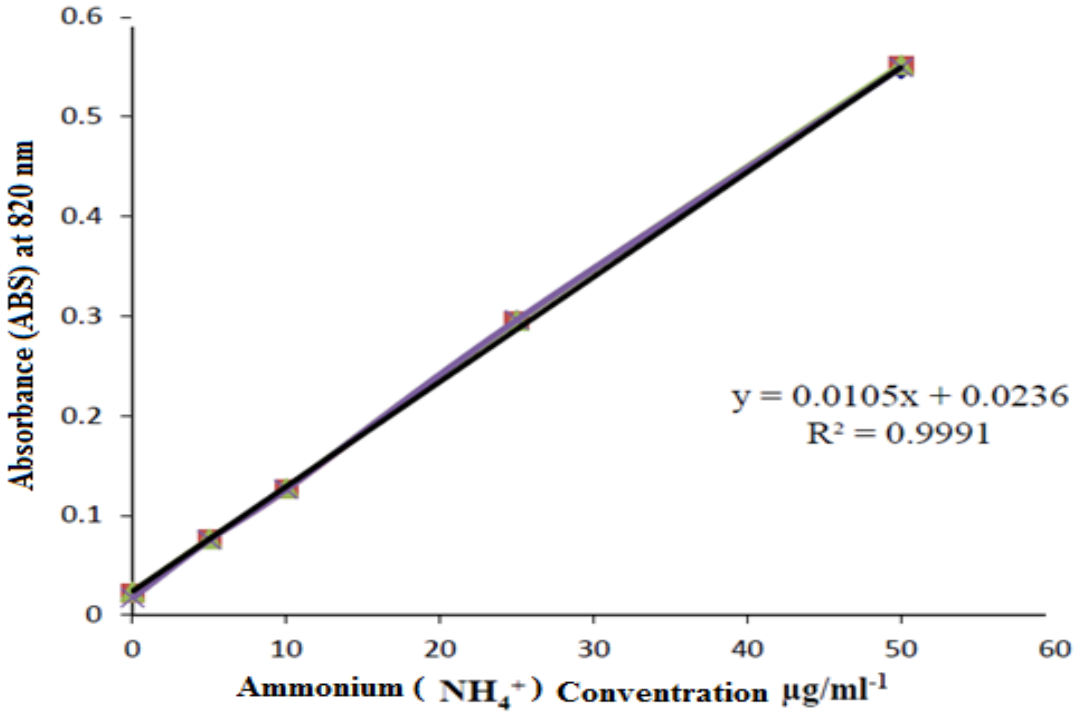
The mixture incubated in water bath for 45 min in 40ºC and read with yellow colour at 820 nm by spectrophotometer (Sims and Grant, 1971).



➢ Standard curves for Ammonium

1. 3.66 g of ammonium sulphate $(NH_4)_2SO_4$ was dissolved in 100 ml distal water dH_2O to obtain 1µg ammonium per µl (mixture 1).
2. 10 ml of mixture 1 into 90 ml of dH_2O to diluted 10 times (mixture 2).
3. Mixture 2 was diluted with dH_2O to produce 0, 10, 20, 40, 60, 80 and 100 µg.

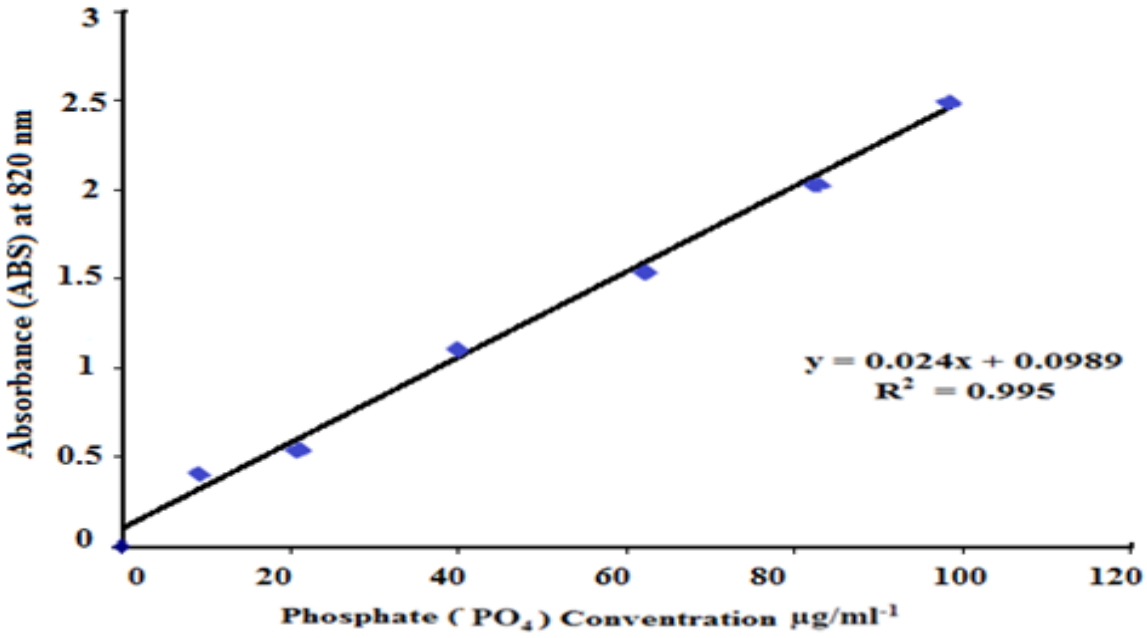
The mixture incubated at 25oC for 20 min in the dark and read with blue colour at 820 nm by spectrophotometer (Wainwright and Pugh, 1973)



- Standard curves for Phosphate.

1. 1.48g Na_2HPO_4 was dissolved into 100 ml dH_2O to obtain 1µg of phosphate per µl (mixture 1).
2. 10 ml of mixture 1 into 90 ml of dH_2O to diluted 10 times (mixture 2).
3. Mixture 2 was diluted with dH_2O to produce 0, 10, 20, 40, 60, 80, and 100 µg.

Then was incubate at 37ºC for 1 hour and read with blue colour at 820 nm by spectrophotometer (Ajaj, 2005)



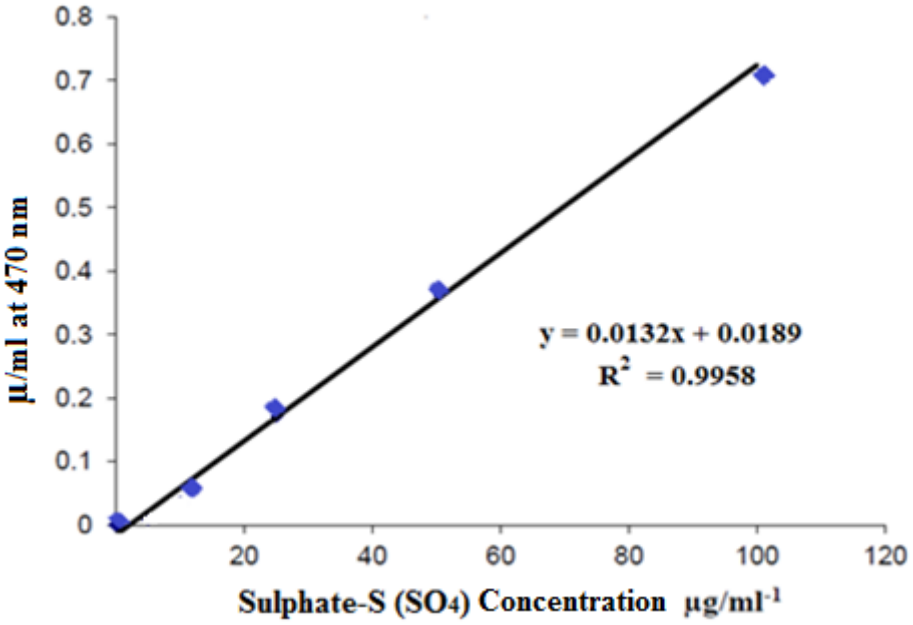
➢ Standard curves for Sulphate-S.

1. 1.47 g $NaSO_4$ was dissolved into 100 ml dH_2O to obtain 1µg of sulphate-S per µl (mixture 1).

2. 10 ml of mixture 1 into 90 ml of dH_2O to diluted 10 times (mixture 2).

3. Mixture 2 was diluted with dH_2O to produce 0, 10, 20, 40, 60, 80, and 100 µg.

Then was incubate at 37ºC for 1 hour and read with yellow colour at 470 nm by spectrophotometer (Hesse, 1971)



(2) Ability of yeast to oxidise elemental sulphur in liquid medium

Figure shows the ability of *Candida rugosa* to oxidise sulphur. This yeast was shown to oxidise inorganic sulphur when growing on a solid medium. As can be seen, *Candida rugosa* biomass was increased to oxidize sulphur which peaked at 0.09 g on week 1. The highest sulphur oxidation by *Candida rugosa* was seen at week 1, there was then a gradual decrease at weeks 2, 3 and 4 in treatment. It can be clearly seen that there was major difference in solubilisation in *Candida inconspicua* the length of the incubation period, where was the highest Sulphate solubilisation at week 2 which peaked at 0.09 g. Then there was a sharp decrease at week 3 then an increase in oxidation at week 4.

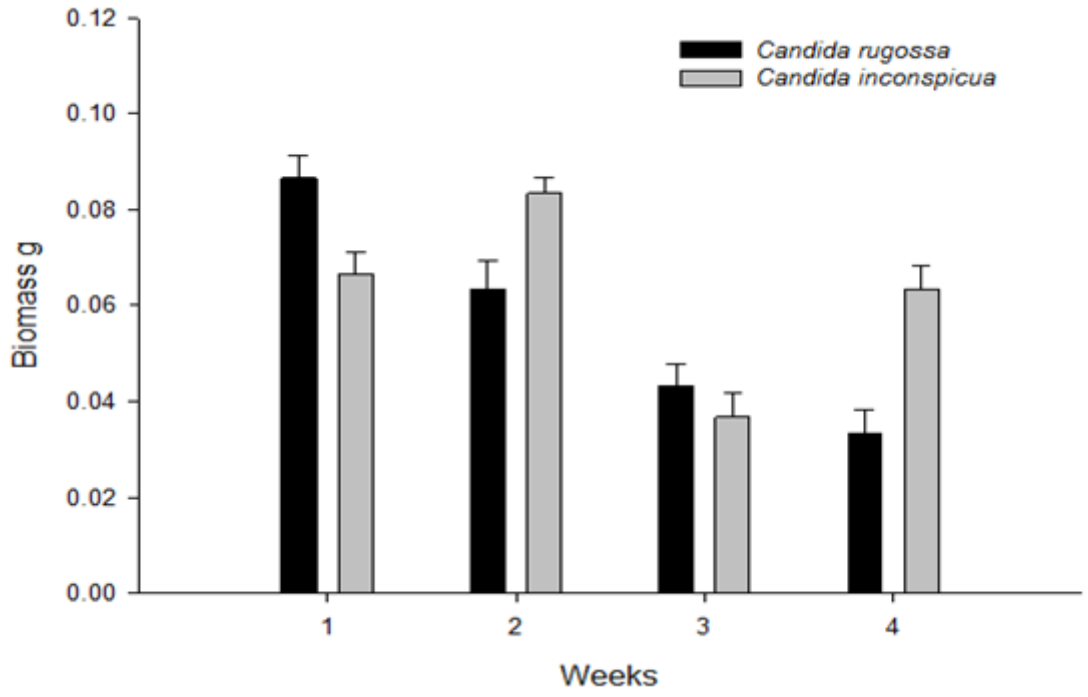


Figure : Yeast biomass during sulphur oxidation *Candida rugosa* *Candida inconspicua.*

(3) Ability of yeast to solubilize insoluble phosphates in liquid medium

Figure shows the ability of *Candida rugosa* to solubilize insoluble phosphate (treatment). Thus, yeast can solubilise phosphates on solid medium. As can be seen, *Candida rugosa* biomass was increased to solubilise insoluble phosphate which peaked at 0.24 g on week 2. The highest phosphate solubilisation in *Candida rugosa* was seen at weeks 2, 1 and 3, there was then a gradual decrease at week 4 in treatment. While, It can be clearly seen that there was major difference in solubilisation in *Candida inconspicua* the length of the incubation period between the weeks Where was the highest phosphate solubilisation at week 2 which peaked at 0.21 g. Hattori, (1973); Alexander, (1977); Paul and Clark, (1996) showed that phosphate solubilising microorganisms solubilise insoluble phosphates by the production of inorganic as well organic acids (e.g. alpha keto-gluconic acid) with a resultant decrease in medium pH .

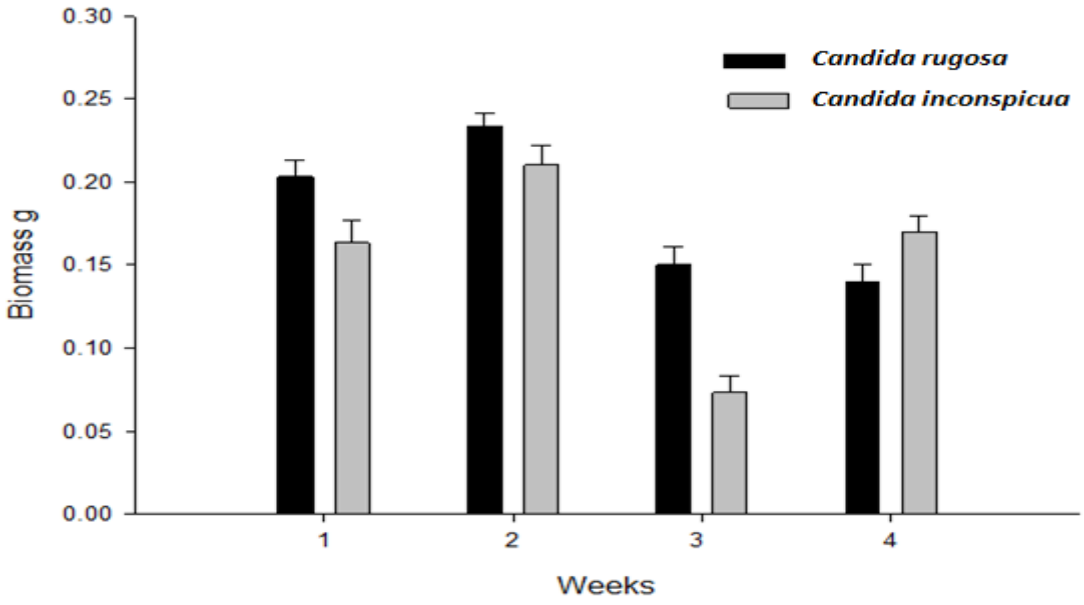


Figure: Yeast biomass production during phosphate solubilisation *Candida rugosa* *Candida inconspicua.*

(4) Statistics

All observations were presented as Mean ± SE (Standard error). The data was analyzed by Sigma Plot© (Version11.0) $P < 0.05$ was considered as significant. Paired two or three samples t-test was performed to check whether means were significantly different.

a) Urea hydrolysis to Ammonium by yeast

1. **Data source:** Data 4 in Urea hydrolysis to Ammonium ……7 Days.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	26.879	0.479	0.277
Row 2	3	0	26.276	1.602	0.925
Row 3	3	0	26.435	1.225	0.707

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.587	0.293	0.205	0.820
Residual	6	8.593	1.432		
Total	8	9.179			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.820).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

2. **Data source:** Data 6 in Urea hydrolysis to Ammonium ……14 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	29.451	2.098	1.211
Row 2	3	0	27.737	0.634	0.366
Row 3	3	0	29.133	0.814	0.470

Source of Variation	DF	SS	MS	F	P
Between Groups	2	4.991	2.495	1.369	0.324
Residual	6	10.933	1.822		
Total	8	15.923			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.324).

Power of performed test with alpha = 0.050: 0.086

The power of the performed test (0.086) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

3. **Data source:** Data 8 in Urea hydrolysis to Ammonium ……21 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	32.883	9.377	5.414

Row 2	3	0	27.295	1.953	1.127
Row 3	3	0	27.549	0.978	0.565

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	2	59.727	29.863	0.966	0.433
Residual	6	185.402	30.900		
Total	8	245.129			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.433).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

4. **Data source:** Data 10 in Urea hydrolysis to Ammonium ……28 Day.

One Way Analysis of Variance

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Row 1	3	0	20.089	10.721	6.190
Row 2	3	0	25.263	15.618	9.017
Row 3	3	0	24.057	17.225	9.945

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	2	43.979	21.989	0.101	0.906
Residual	6	1311.073	218.512		
Total	8	1355.051			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.906).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

b) Oxidation of Ammonium to Nitrate by yeast

1. **Data source:** Data 8 in Oxidation of Ammonium to Nitrate …7 Days.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	21.940	8.372	4.834
Row 2	3	0	19.407	9.603	5.544
Row 3	3	0	20.207	7.177	4.143

Source of Variation	DF	SS	MS	F	P
Between Groups	2	10.062	5.031	0.0706	0.933
Residual	6	427.633	71.272		
Total	8	437.696			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.933).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

2. **Data source:** Data 6 in Oxidation of Ammonium to Nitrate 14 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	52.007	18.415	10.632
Row 2	3	0	44.240	10.184	5.880
Row 3	3	0	26.640	0.854	0.493

Source of Variation	DF	SS	MS	F	P
Between Groups	2	1013.549	506.774	3.428	0.102
Residual	6	887.087	147.848		
Total	8	1900.636			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.102).

Power of performed test with alpha = 0.050: 0.312

The power of the performed test (0.312) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

3. **Data source:** Data 11 in Oxidation of Ammonium to Nitrate 21 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	33.307	13.834	7.987
Row 2	3	0	42.807	16.919	9.768
Row 3	3	0	53.140	3.857	2.227

Source of Variation	DF	SS	MS	F	P
Between Groups	2	590.389	295.194	1.798	0.244
Residual	6	985.033	164.172		
Total	8	1575.422			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.244).

Power of performed test with alpha = 0.050: 0.130

The power of the performed test (0.130) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

4. **Data source:** Data 13 in Oxidation of Ammonium to Nitrate 28 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	7.807	2.627	1.517
Row 2	3	0	30.007	6.408	3.700

Row 3	3	0	33.540	6.986	4.034

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	2	1167.529	583.764	18.096	0.003
Residual	6	193.553	32.259		
Total	8	1361.082			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.003).

Power of performed test with alpha = 0.050: 0.983

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor:

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
Row 3 vs. Row 1	25.733	5.549	0.004	Yes
Row 2 vs. Row 1	22.200	4.787	0.006	Yes
Row 3 vs. Row 2	3.533	0.762	0.475	No

c) Oxidation of Sulphur by yeast.

1. **Data source:** Data 4 in Oxidation of Sulphur ……7 Days.

One Way Analysis of Variance

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Row 1	3	0	0.423	0.0252	0.0145
Row 2	3	0	0.520	0.243	0.140

Row 3	3	0	1.193	0.112	0.0644

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1.056	0.352	14.165	0.001
Residual	8	0.199	0.0248		
Total	11	1.254			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.820).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

2. **Data source:** Data 6 in Oxidation of Sulphur ……14 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	0.260	0.0265	0.0153
Row 2	3	0	0.200	0.0819	0.0473
Row 3	3	0	0.777	0.156	0.0899

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.620	0.207	18.209	<0.001
Residual	8	0.0907	0.0113		

Total	11	0.710

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.324).

Power of performed test with alpha = 0.050: 0.086

The power of the performed test (0.086) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

3. **Data source:** Data 8 in Oxidation of Sulphur ……21 Day.

One Way Analysis of Variance

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Row 1	3	0	3.667	1.528	0.882
Row 2	3	0	3.667	0.577	0.333
Row 3	3	0	5.000	1.000	0.577

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	3	4.000	1.333	1.333	0.330
Residual	8	8.000	1.000		
Total	11	12.000			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.433).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

4. **Data source:** Data 10 in Oxidation of Sulphur ……28 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	20.089	10.721	6.190
Row 2	3	0	25.263	15.618	9.017
Row 3	3	0	24.057	17.225	9.945

Source of Variation	DF	SS	MS	F	P
Between Groups	2	43.979	21.989	0.101	0.906
Residual	6	1311.073	218.512		
Total	8	1355.051			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.906).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

d) phosphate solubilisation by yeasts

1. **Data source:** Data 8 in phosphate solubilisation …7 Days.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	21.940	8.372	4.834
Row 2	3	0	19.407	9.603	5.544
Row 3	3	0	20.207	7.177	4.143

Source of Variation	DF	SS	MS	F	P
Between Groups	2	10.062	5.031	0.0706	0.933
Residual	6	427.633	71.272		
Total	8	437.696			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.933).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

2. **Data source:** Data 6 in phosphate solubilisation 14 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	52.007	18.415	10.632
Row 2	3	0	44.240	10.184	5.880
Row 3	3	0	26.640	0.854	0.493

Source of Variation	DF	SS	MS	F	P
Between Groups	2	1013.549	506.774	3.428	0.102
Residual	6	887.087	147.848		
Total	8	1900.636			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.102).

Power of performed test with alpha = 0.050: 0.312

The power of the performed test (0.312) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

3. **Data source:** Data 11 in phosphate solubilisation 21 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	33.307	13.834	7.987
Row 2	3	0	42.807	16.919	9.768
Row 3	3	0	53.140	3.857	2.227

Source of Variation	DF	SS	MS	F	P
Between Groups	2	590.389	295.194	1.798	0.244
Residual	6	985.033	164.172		
Total	8	1575.422			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.244).

Power of performed test with alpha = 0.050: 0.130

The power of the performed test (0.130) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

4. **Data source:** Data 13 in phosphate solubilisation 28 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	7.807	2.627	1.517
Row 2	3	0	30.007	6.408	3.700
Row 3	3	0	33.540	6.986	4.034

Source of Variation	DF	SS	MS	F	P
Between Groups	2	1167.529	583.764	18.096	0.003
Residual	6	193.553	32.259		
Total	8	1361.082			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.003).

Power of performed test with alpha = 0.050: 0.983

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
Row 3 vs. Row 1	25.733	5.549	0.004	Yes
Row 2 vs. Row 1	22.200	4.787	0.006	Yes
Row 3 vs. Row 2	3.533	0.762	0.475	No

e) Measuring of pH

1. **Data source:** Data 3 in Measuring of pH of samples….7 Days.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	9.237	0.0462	0.0267
Row 2	3	0	9.260	0.0400	0.0231
Row 3	3	0	9.200	0.0265	0.0153

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.00549	0.00274	1.857	0.236
Residual	6	0.00887	0.00148		
Total	8	0.0144			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.236).

Power of performed test with alpha = 0.050: 0.136

The power of the performed test (0.136) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

2. **Data source:** Data 5 in Measuring of pH ….14 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	8.917	0.0850	0.0491
Row 2	3	0	8.960	0.0520	0.0300
Row 3	3	0	8.953	0.0289	0.0167

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.00327	0.00163	0.455	0.655
Residual	6	0.0215	0.00359		
Total	8	0.0248			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.655).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

3. **Data source:** Data 6 in Measuring of pH ….21 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	7.323	0.395	0.228
Row 2	3	0	8.657	0.112	0.0644
Row 3	3	0	8.737	0.0702	0.0406

Source of Variation	DF	SS	MS	F	P
Between Groups	2	3.782	1.891	32.728	<0.001
Residual	6	0.347	0.0578		
Total	8	4.128			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
Row 3 vs. Row 1	1.413	7.202	0.001	Yes
Row 2 vs. Row 1	1.333	6.794	<0.001	Yes
Row 3 vs. Row 2	0.0800	0.408	0.698	No

4. **Data source:** Data 4 in Measuring of pH ….28 Day.

One Way Analysis of Variance

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	5.890	0.529	0.305

Row 2	3	0	7.953	0.211	0.122
Row 3	3	0	8.143	0.107	0.0617

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	2	9.371	4.685	41.870	<0.001
Residual	6	0.671	0.112		
Total	8	10.042			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor:

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
Row 3 vs. Row 1	2.253	8.250	<0.001	Yes
Row 2 vs. Row 1	2.063	7.554	<0.001	Yes
Row 3 vs. Row 2	0.190	0.696	0.513	No

APPENDIX: B

(1) Total the number of Colonies of Yeasts and the Weight in cow dung during 24 hours of drying.

Colonies were calculated after drying in room temperature for 24 hours which showed clear evidence of decreased the weight dung and increase number of colonies during length incubation at 28 °C.

Table: Decreased the weight dung after drying in room temperature for 24 hours during length incubation at 28 °C.

Time	**Weight the dung**
0 Time	15 g
5 Hours	13.75 g
10 Hours	11.29 g
15 Hours	7.95 g
20 Hours	7.09 g
25 Hours	5.64 g

Table: Increase number of colonies after drying in room temperature for 24 hours during length incubation at 28 °C.

Time	**Average number of the Colonies on 3 plates**
0 Time	8.6
5 Hours	11.6
10 Hours	23
15 Hours	127
20 Hours	144
25 Hours	212

The total Yeast count of cow dung after drying in room temperature for 24 hours during length incubation at 28 °C. It can be clearly seen that the numbers of colony forming units in the dung was sharply increased during length incubation.

(2) Weight the dung during 24 hours of drying in room temperature.

The results reveal the amount of weight dung degraded during length incubation at 28 °C. In spite of weight degraded were identified increased of colony forming units in the dung, a linear correlation was observed between the numbers of colony and weight losses, could be attributed to the fact that it forms spores which help microorganisms to withstand harsh conditions, such as sun drying to their ability to grow and survive in these environments.

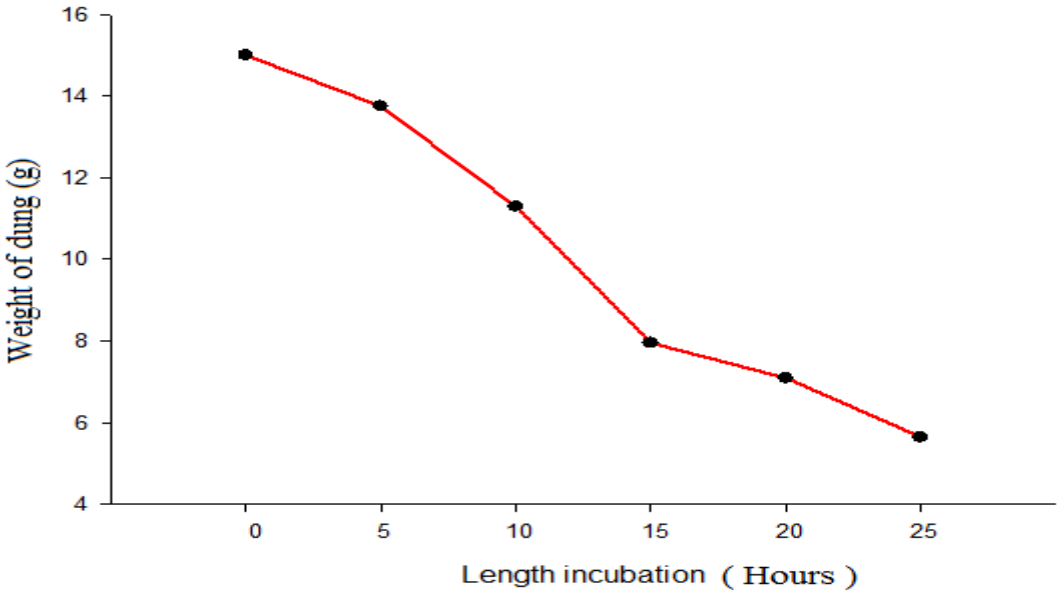


Figure: Decreased the weight dung after drying in room temperature for 24 hours during length incubation at 28 °C.

(3) Total the number of Colonies of Yeasts in dung during 24 hours of drying.

The total Fungal count of cow dung after drying in room temperature for 24 hours during length incubation at 28 °C. It can be clearly seen that the numbers of colony forming units in the dung was sharply increased during length incubation.

This will obviously relate to their ability to grow and survive in these environments in soils and cow dung due to has the ability to form biofilms on the surfaces of both organic and inorganic materials. Fungal cells grow and reproduce with sufficient nutrients and oxygen (Sena *et al.*, 2006, Buck, 1990).

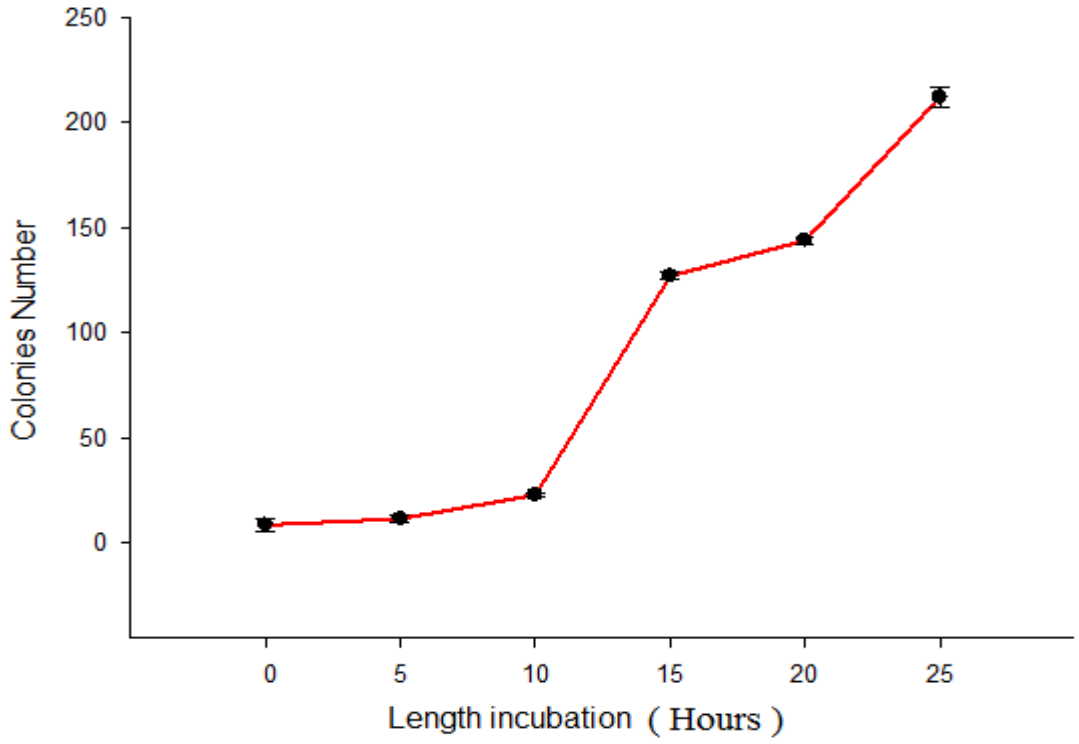


Figure: Increase number of colonies after drying in room temperature for 24 hours during length incubation at 28 °C.

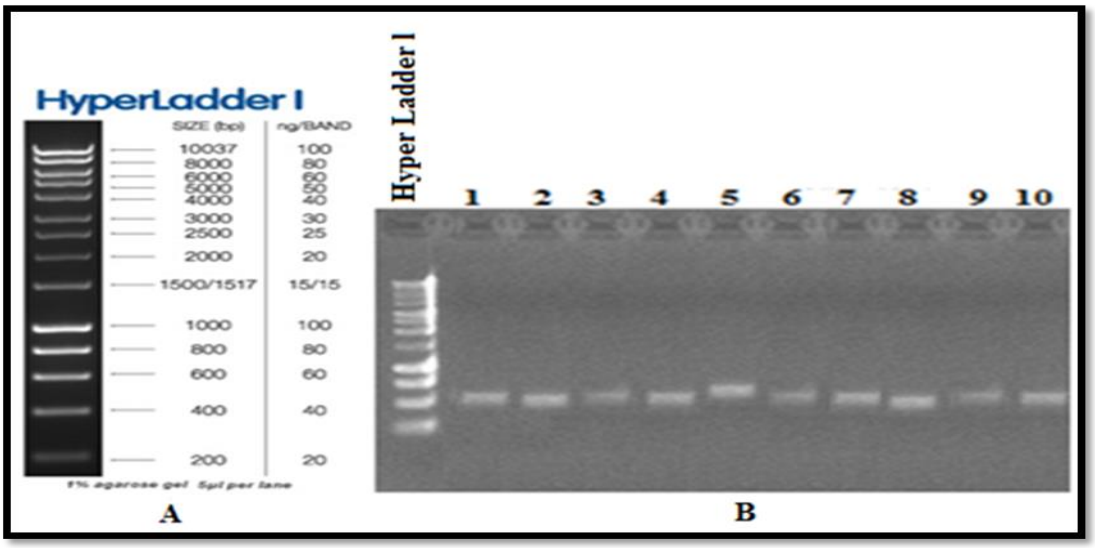
APPENDIX: C

PCR amplification of extracted DNA.

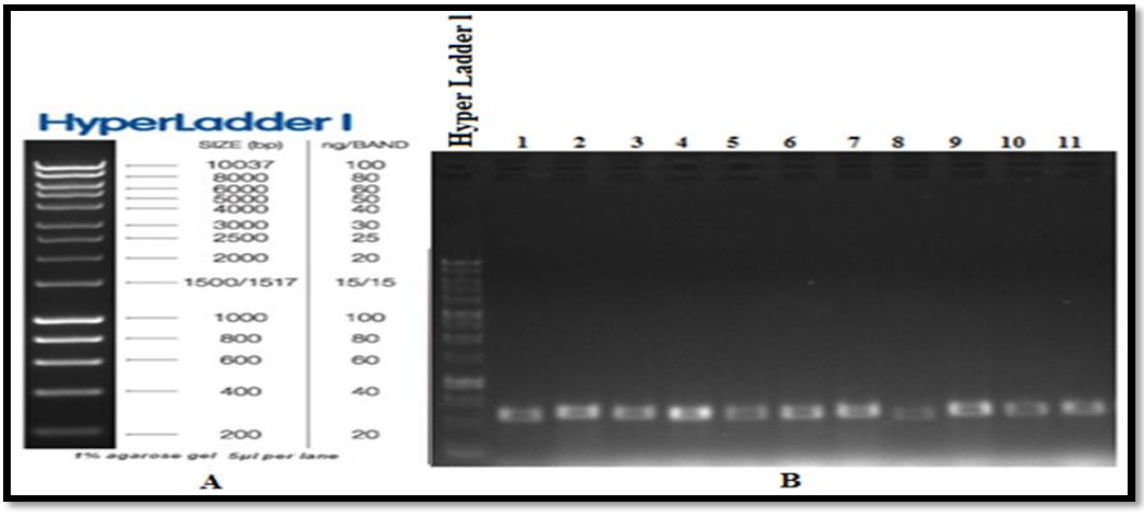
A) Standard Hyperladder I produces of 14 regularly spaced bands and each lane (5 µl) provides 720 ng of DNA.

B) Successful genomic DNA amplification after finishing the DNA extraction processes by using 1% agarose gel electrophoresis for 40 minutes at 85 V.

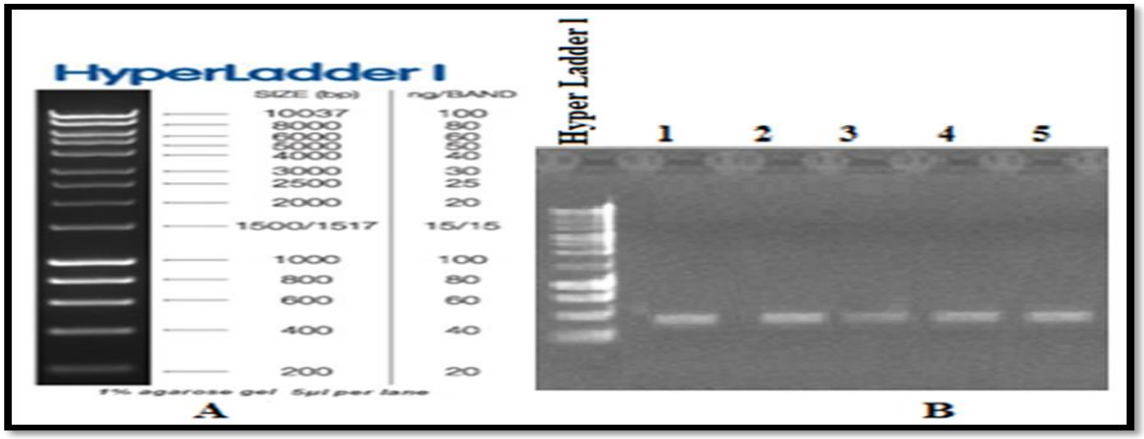
(1) PCR amplification of extracted DNA of Yeasts from various sinks.



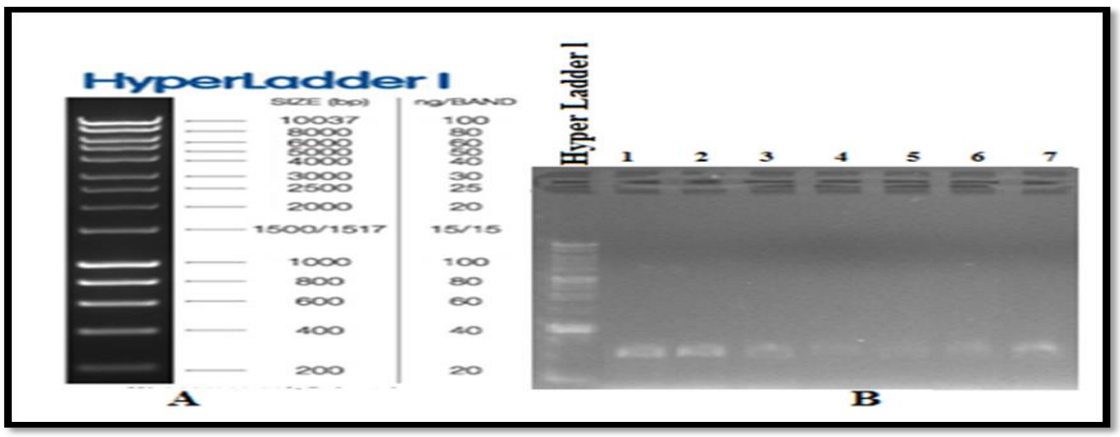
(2) PCR amplification of extracted DNA of Yeasts from various computer keyboards.



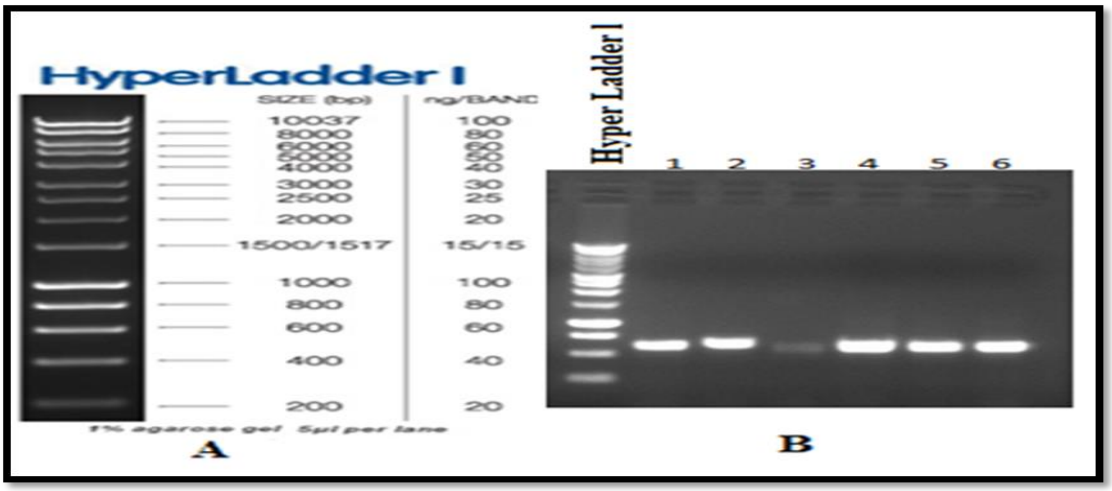
(3) PCR amplification of extracted DNA of Yeasts from various Mobile devices.



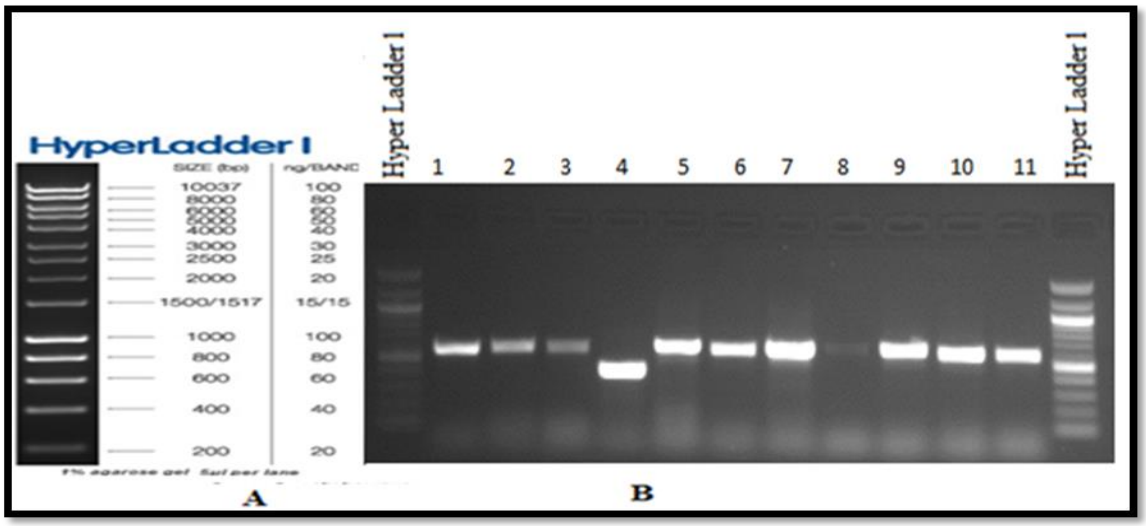
(4) PCR amplification of extracted DNA of Yeasts from hand warm air dryers.



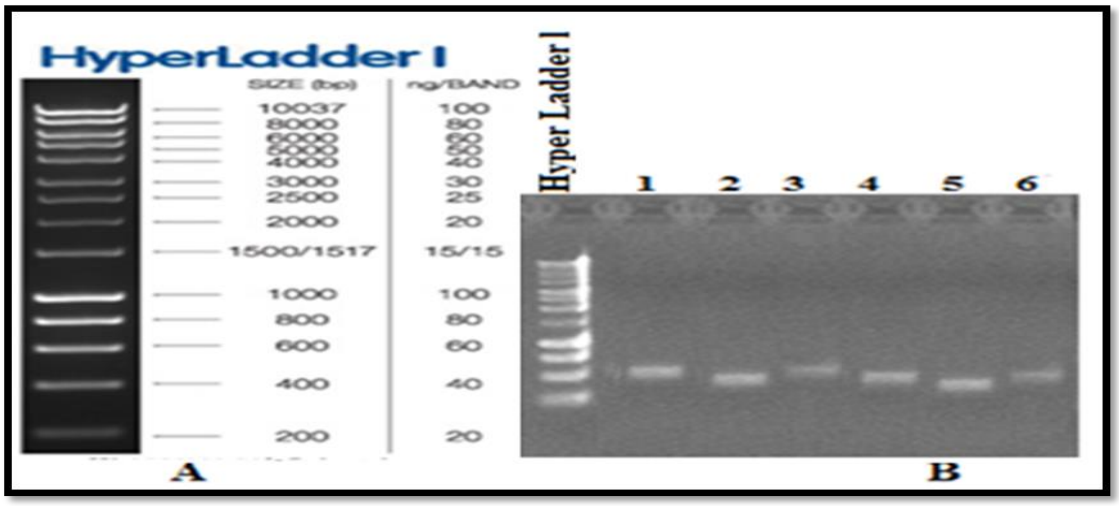
(5) PCR amplification of extracted DNA of Yeasts from various vacuum cleaners.



(6) PCR amplification of extracted DNA of Yeasts from the soles of a various shoes.



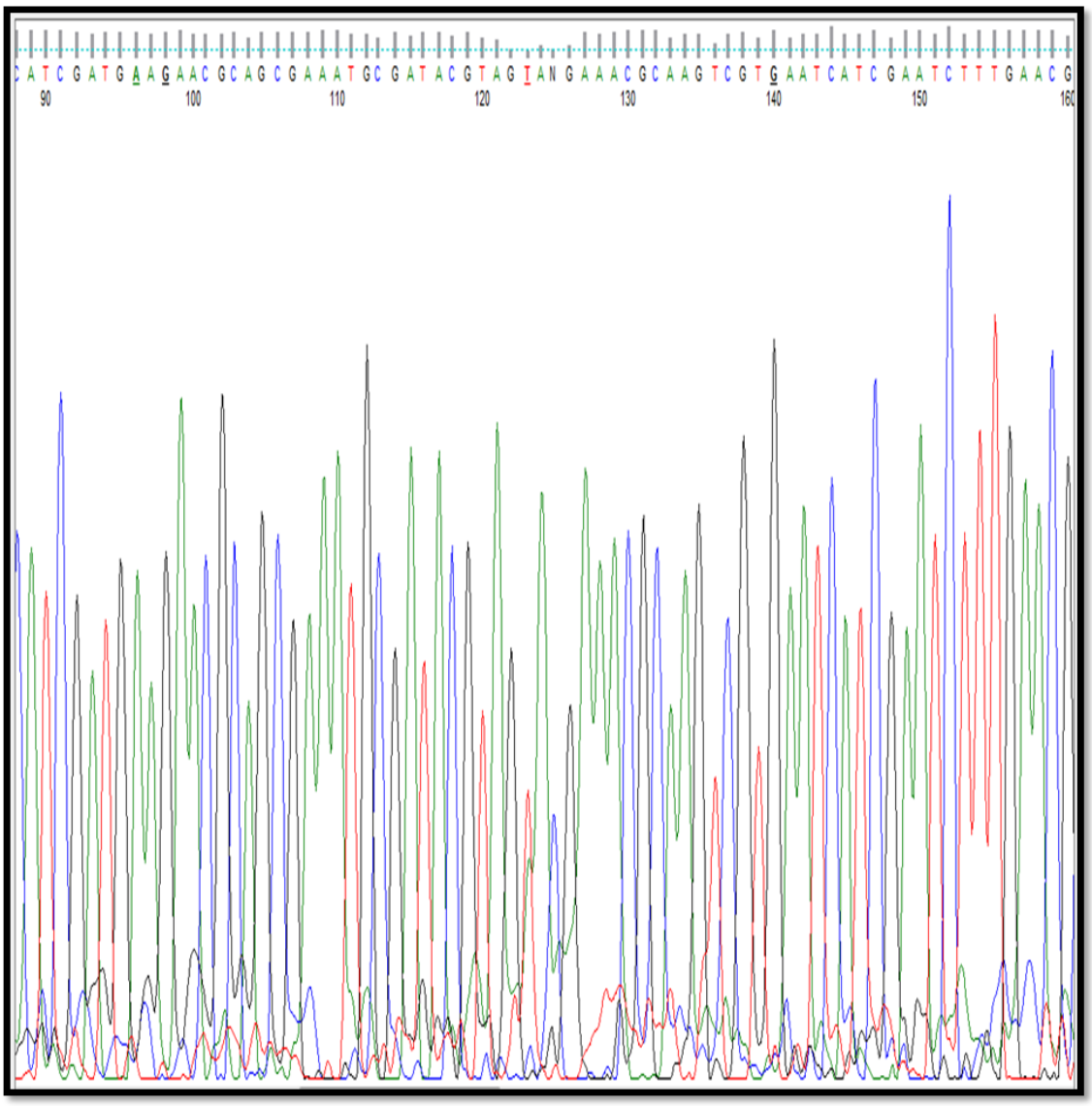
(7) PCR amplification of extracted DNA of Yeasts from used Toothbrushes.



APPENDIX: D

Result of samples after sent to the University of Sheffield Medical School Core Genetics Unit, for further sequencing as follows (Phylogenetic analysis of yeast Isolated):

- ***Candida rugose***.



Candida rugosa voucher UFLA FFT1.4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: gb|KM368814.1| Length: 346 Number of Matches: 1

Related Information

Range 1: 79 to 322 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
419 bits(464)	1e-113	239/244(98%)	0/244(0%)	Plus/Plus

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Query  1    TAATTCAAAACTTTCAACAACGGATCTCTAGGTTCTCGCATCGATGAAGAACGCAGCGAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  79   TAATTCAAAACTTTCAACAACGGATCTCTAGGTTCTCGCATCGATGAAGAACGCAGCGAA  138

Query  61   ATGCGATACGTAGTANGAAACGCAAGTCGTGAATCATCGAATCTTTGAACGCACATTGCG  120
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  139  ATGCGATACGTAGTACGAAACGCAAGTCGTGAATCATCGAATCTTTGAACGCACATTGCG  198

Query  121  CTGTGTGGCATTCCACACAGCATGCCTGTTTGAGCAATATTTCTCTCTCGCAAGGTGTTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  199  CTGTGTGGCATTCCACACAGCATGCCTGTTTGAGCAATATTTCTCTCTCGCAAGGTGTTG  258

Query  181  GGCACCACGCCGGCAGGGGTCTGCCAGAAACGAATTTGTCAAAAACAGTTAAGCTTGTTA  240
            |||||||||||||||| ||||||||||||||| ||||| ||||||||||||||||||||
Sbjct  259  GGCACCACGCCGGCAGGCGTCTGCCAGAAACGAAATTGTCTAAAACAGTTAAGCTTGTTA  318

Query  241  AAGA  244
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Sbjct  319  CAGA  322
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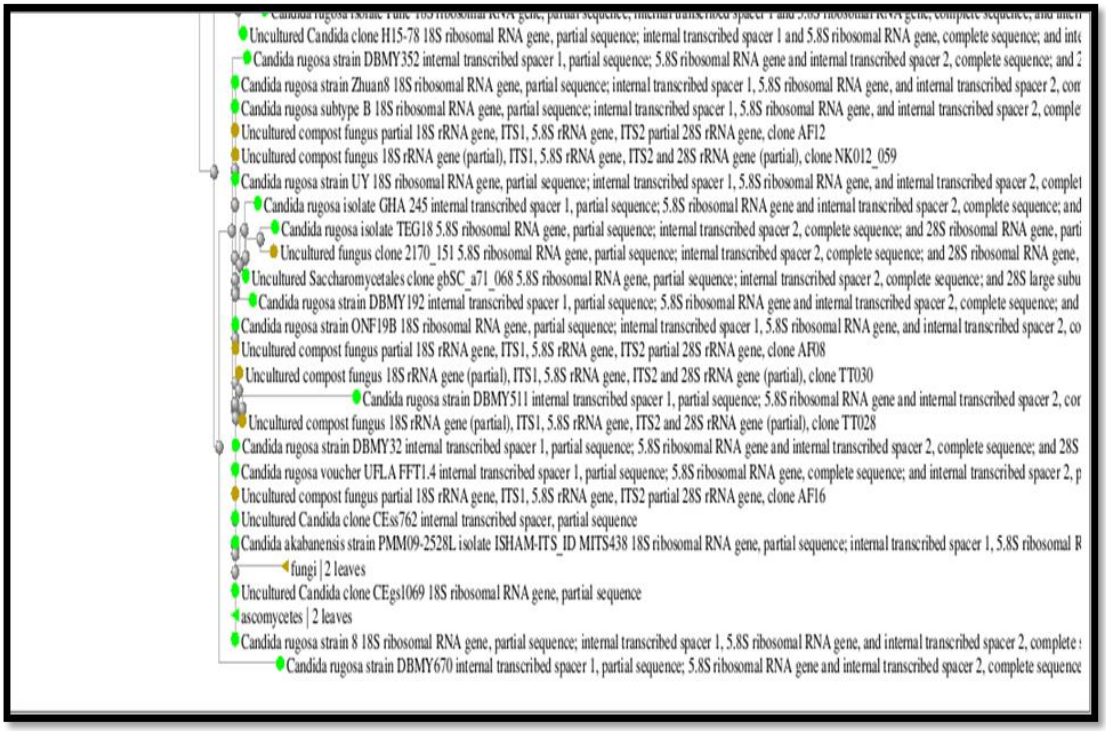
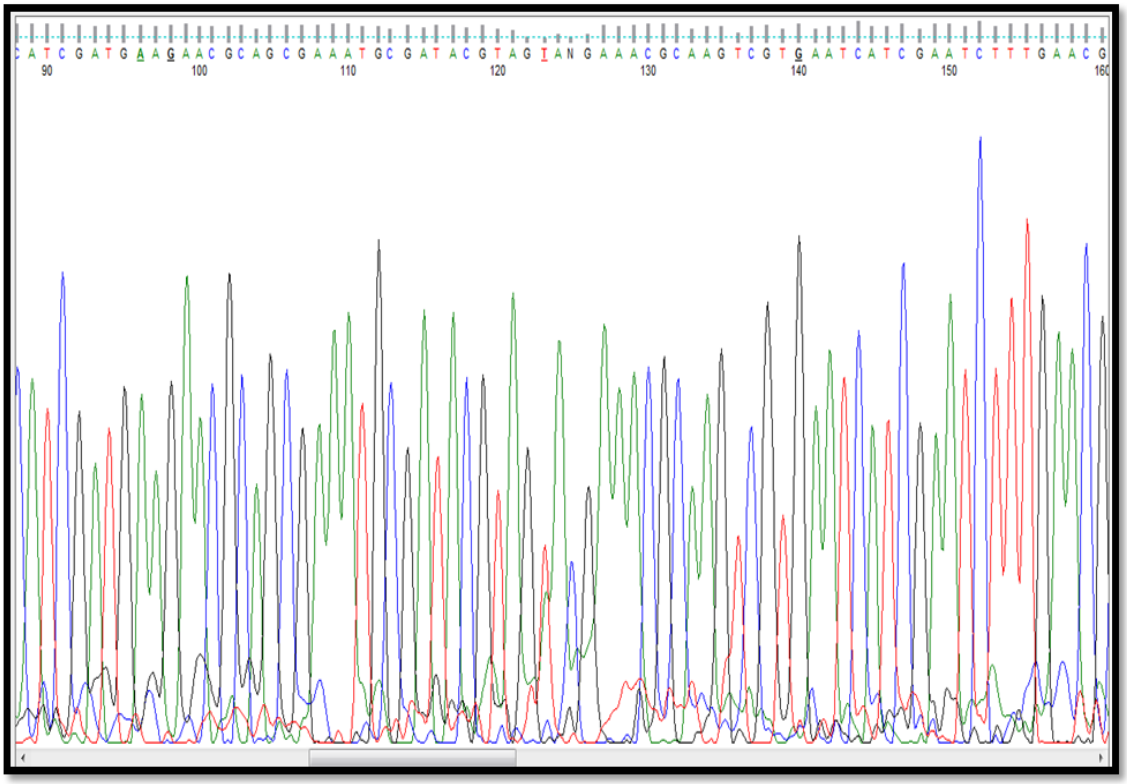


- ***Candida inconspicua***



Candida inconspicua isolate B14 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

Sequence ID: gb|EU315758.1| Length: 518 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
435 bits(482)	6e-119	241/241(100%)	0/241(0%)	Plus/Plus

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Query  1    GAAAACAAAAACACCTAAAATGTGGAATATAGCATATAGTCGACAAGAGAAATCTACGAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  70   GAAAACAAAAACACCTAAAATGTGGAATATAGCATATAGTCGACAAGAGAAATCTACGAA  129

Query  61   AAACAAACAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAGCG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  130  AAACAAACAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAGCG  189

Query  121  AAATGCGATACCTAGTGTGAATTGCAGCCATCGTGAATCATCGAGTTCTTGAACGCACAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  190  AAATGCGATACCTAGTGTGAATTGCAGCCATCGTGAATCATCGAGTTCTTGAACGCACAT  249

Query  181  TGCGCCCCTCGGCATTCCGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTGCGCGTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  TGCGCCCCTCGGCATTCCGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTGCGCGTG  309

Query  241  C  241
            |
Sbjct 310    C  310
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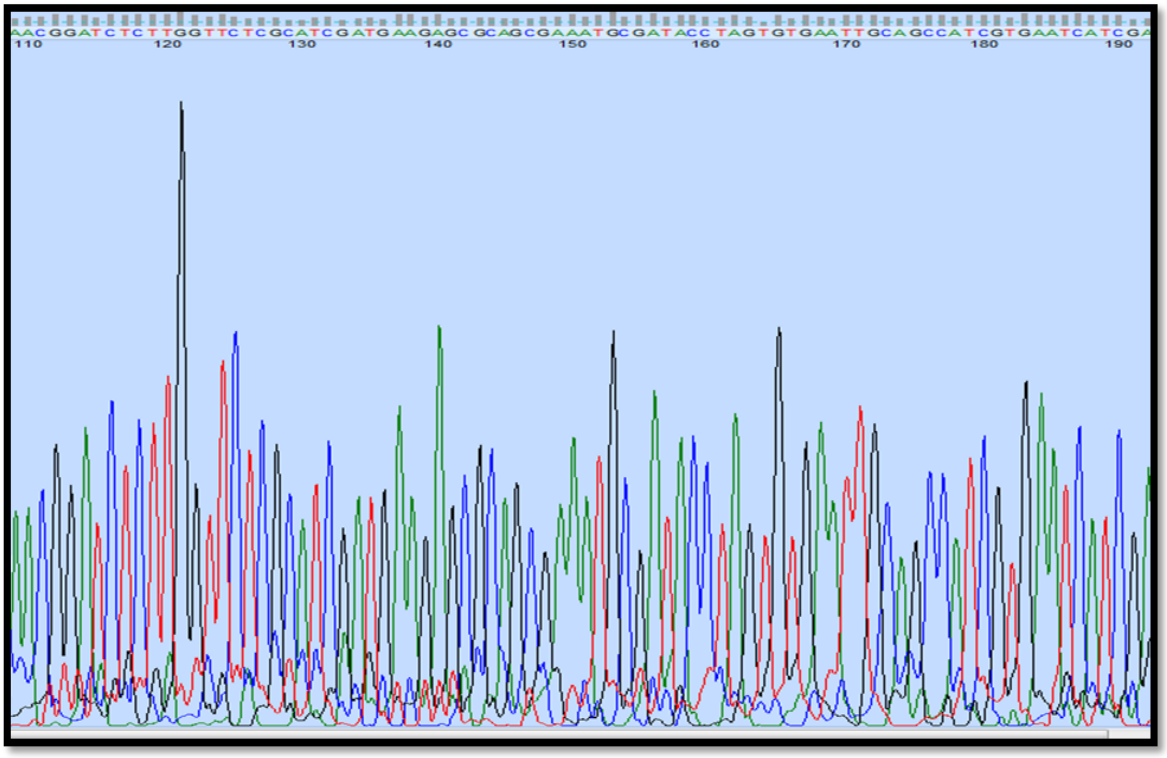
- ***Candida xylopsoci***



Candida xylopsoci 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), strain WM 07.10

Sequence ID: emb|FM178339.1| Length: 532 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
453 bits(502)	3e-124	251/251(100%)	0/251(0%)	Plus/Plus

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Query  1    AAAACAAAAACACCTAAAATGTGGAATATAGCATATAGTCGACAAGAGAAATCTACGAAA  60
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Sbjct  74   AAAACAAAAACACCTAAAATGTGGAATATAGCATATAGTCGACAAGAGAAATCTACGAAA  133

Query  61   AACAAACAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAGCGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  134  AACAAACAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAGCGA  193

Query  121  AATGCGATACCTAGTGTGAATTGCAGCCATCGTGAATCATCGAGTTCTTGAACGCACATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  194  AATGCGATACCTAGTGTGAATTGCAGCCATCGTGAATCATCGAGTTCTTGAACGCACATT  253

Query  181  GCGCCCCTCGGCATTCCGGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTGCGCGTGC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  254  GCGCCCCTCGGCATTCCGGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTGCGCGTGC  313

Query  241  GCAGAGTTGGG  251
            ||||||||||
Sbjct  314  GCAGAGTTGGG  324
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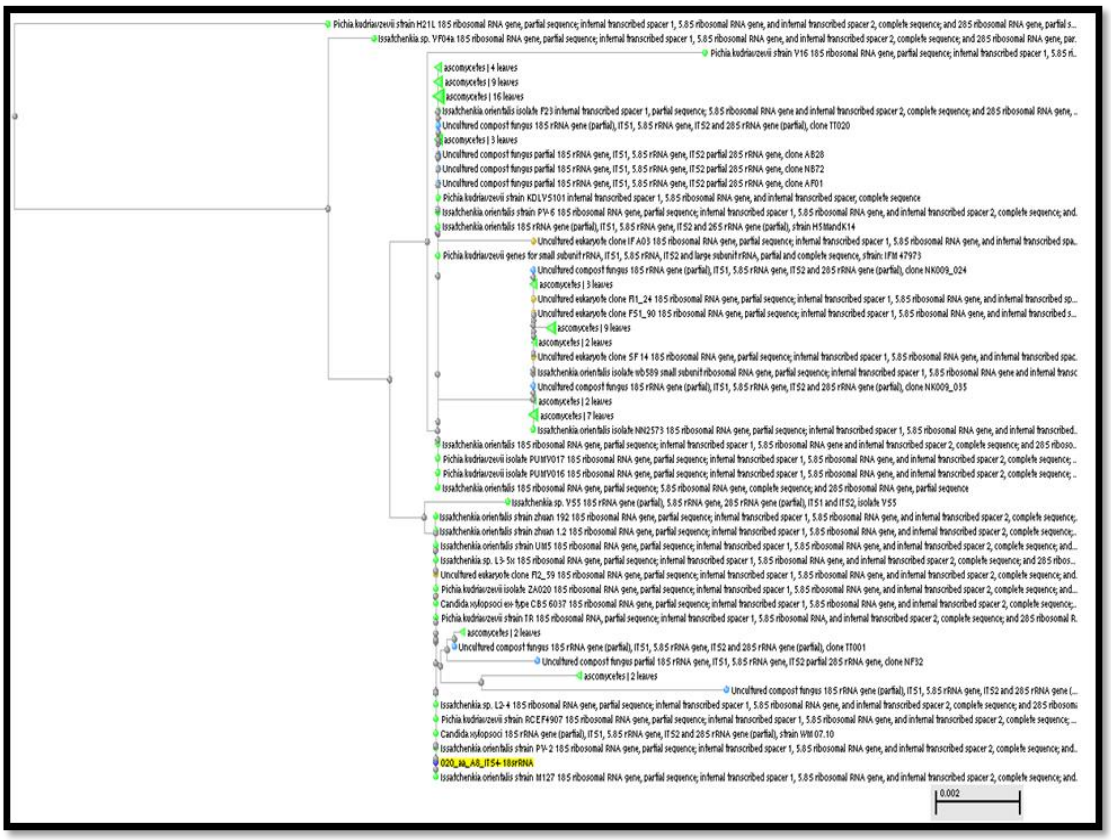
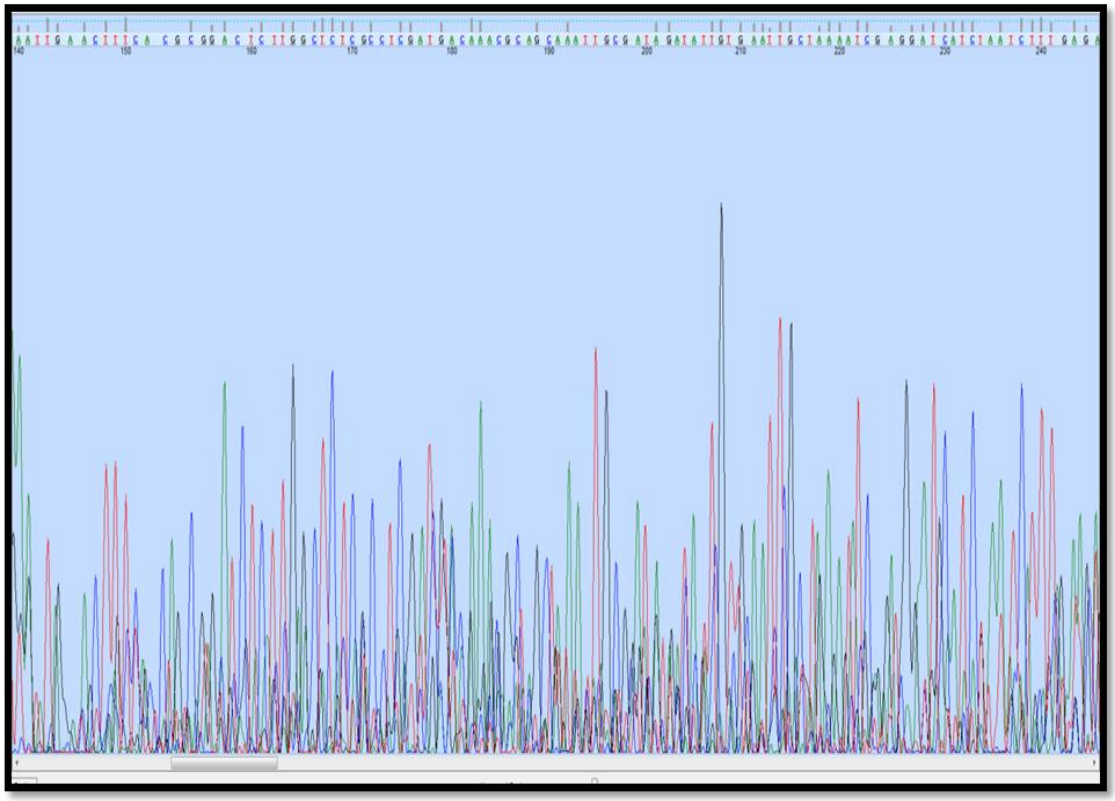


- ***Rhodotorula mucilaginosa***



Score	Expect	Identities	Gaps	Strand
237 bits(262)	7e-59	215/260(83%)	12/260(4%)	Plus/Plus

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Query  72   TGAAAGTATTAA--TTTATAA--AAATTGAACTTTCACG--CGGA-CTCTTGGCTCTCGC  124
           |||| |||||||  |||||||  ||||  ||||||||    |||| ||||||||||||||
Sbjct  107  TGAATGTATTAAATTTTATAACAAAATAAAACTTTCAACAACGGATCTCTTGGCTCTCGC  166

Query  125  CTCGATGACAAACGCAGCAAATTGCGATAGATATTGTGAATTGCTAAAATC-GAGGATCA  183
            |||||||  |||||||| || |||||||  || ||||||||||  || || | | ||||
Sbjct  167  ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA  226

Query  184  TCTAATCTTTGAGA-CACCTTGCTCGCCATGGTATTCCATGGAGCA-GCCTGGTTGA-TG  240
            || |||||||||   |||||||| | |||||||||||| ||||||| ||||| |||| ||
Sbjct  227  TCGAATCTTTGAACGCACCTTGCGCTCCATGGTATTCCGTGGAGCATGCCTGTTTGAGTG  286

Query  241  GCGTGAAGACTTCAACCCTCCTCTTTCT-AATGATTGGAGATGTGGTTGGTTTCTGAGCG  299
             | |||| ||||||||||||||||||||| |||||||| ||| ||| ||||||||||||||
Sbjct  287  TCATGAATACTTCAACCCTCCTCTTTCTTAATGATTGAAGAGGTGTTTGGTTTCTGAGCG  346

Query  300  CTGCTGGTCTTTAAGATCTA  319
            ||||||| ||||||| ||||
Sbjct  347  CTGCTGGCCTTTAAGGTCTA  366
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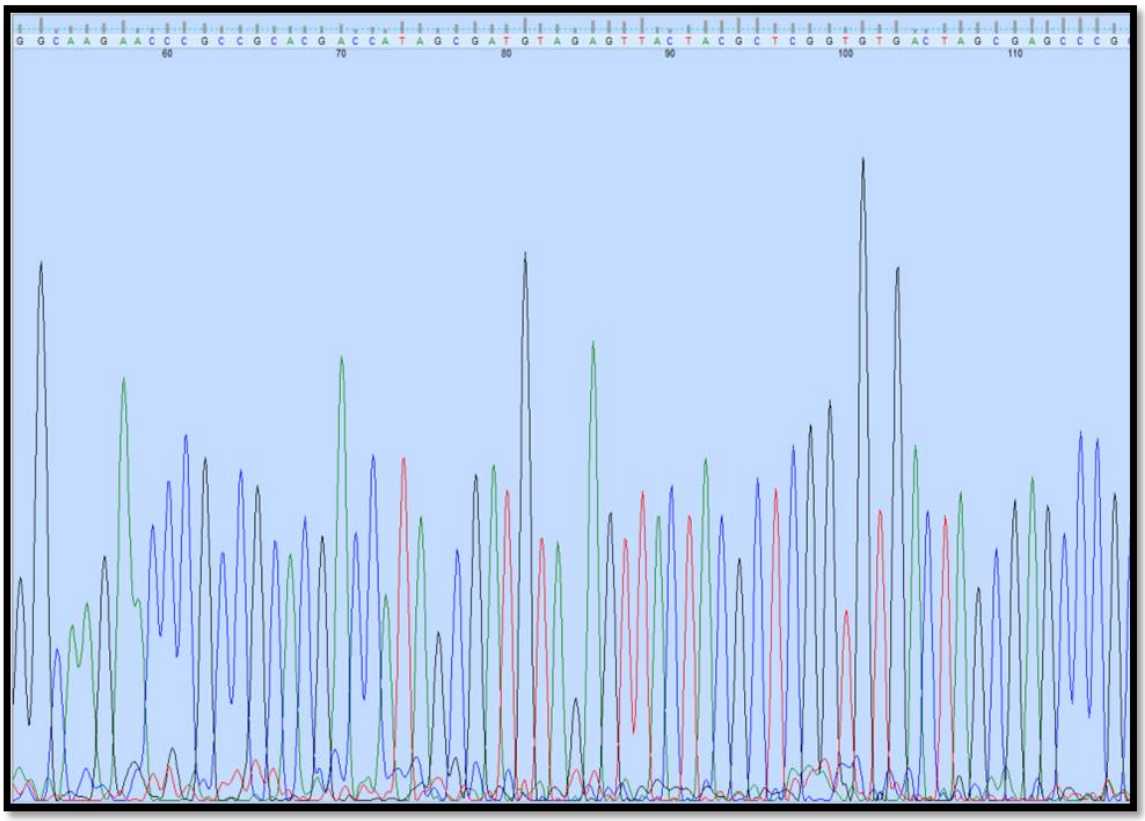
- ***Neurospora tetrasperma***



Neurospora tetrasperma strain XF35 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

Sequence ID: gb|JX136749.1|Length: 587Number of Matches: 1

Related Information

Range 1: 8 to 507GenBankGraphics

Score	Expect	Identities	Gaps	Strand
902 bits(1000)	0.0	500/500(100%)	0/500(0%)	Plus/Minus

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Query  1    GGCAAGAACCCGCCGCACGACCATAGCGATGTAGAGTTACTACGCTCGGTGTGACTAGCG  60
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Sbjct  507  GGCAAGAACCCGCCGCACGACCATAGCGATGTAGAGTTACTACGCTCGGTGTGACTAGCG  448

Query  61   AGCCCGCCACTGATTTTGAGGGACCGCGGGCAGCCGCGGATCCCCAACGCAAGCAGAGCT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  447  AGCCCGCCACTGATTTTGAGGGACCGCGGGCAGCCGCGGATCCCCAACGCAAGCAGAGCT  388

Query  121  TGATGGTTGAAATGACGCTCGAACAGGCATGCTCGCCAGAATACTGGCGAGCGCAATGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387  TGATGGTTGAAATGACGCTCGAACAGGCATGCTCGCCAGAATACTGGCGAGCGCAATGTG  328

Query  181  CGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  327  CGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCT  268

Query  241  GCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGACTTATTTAAA  300
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Sbjct  267  GCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGACTTATTTAAA  208

Query  301  AGTTTACTCAGAGAGACATAAAATATCAAGAGTTTAGTTTCGGCACTCCGGCGGGCAGCC  360
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Sbjct  207  AGTTTACTCAGAGAGACATAAAATATCAAGAGTTTAGTTTCGGCACTCCGGCGGGCAGCC  148

Query  361  TCCCGCGAGCGGGAGACCCGAGGATCCGGGAGGACCCGAGGGCCTTTCCGGACCGCCAGC  420
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Sbjct  147  TCCCGCGAGCGGGAGACCCGAGGATCCGGGAGGACCCGAGGGCCTTTCCGGACCGCCAGC  88

Query  421  GCCGAGGCAACCGTACGGGTAAGATTCGCGATGGTTTGTGGGAGTTTTGCAACTCTGTAA  480
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Sbjct  87   GCCGAGGCAACCGTACGGGTAAGATTCGCGATGGTTTGTGGGAGTTTTGCAACTCTGTAA  28

Query  481  TGATCCCTCCGCAGGTTCAC  500
            ||||||||||||||||||||
Sbjct  27   TGATCCCTCCGCAGGTTCAC  8
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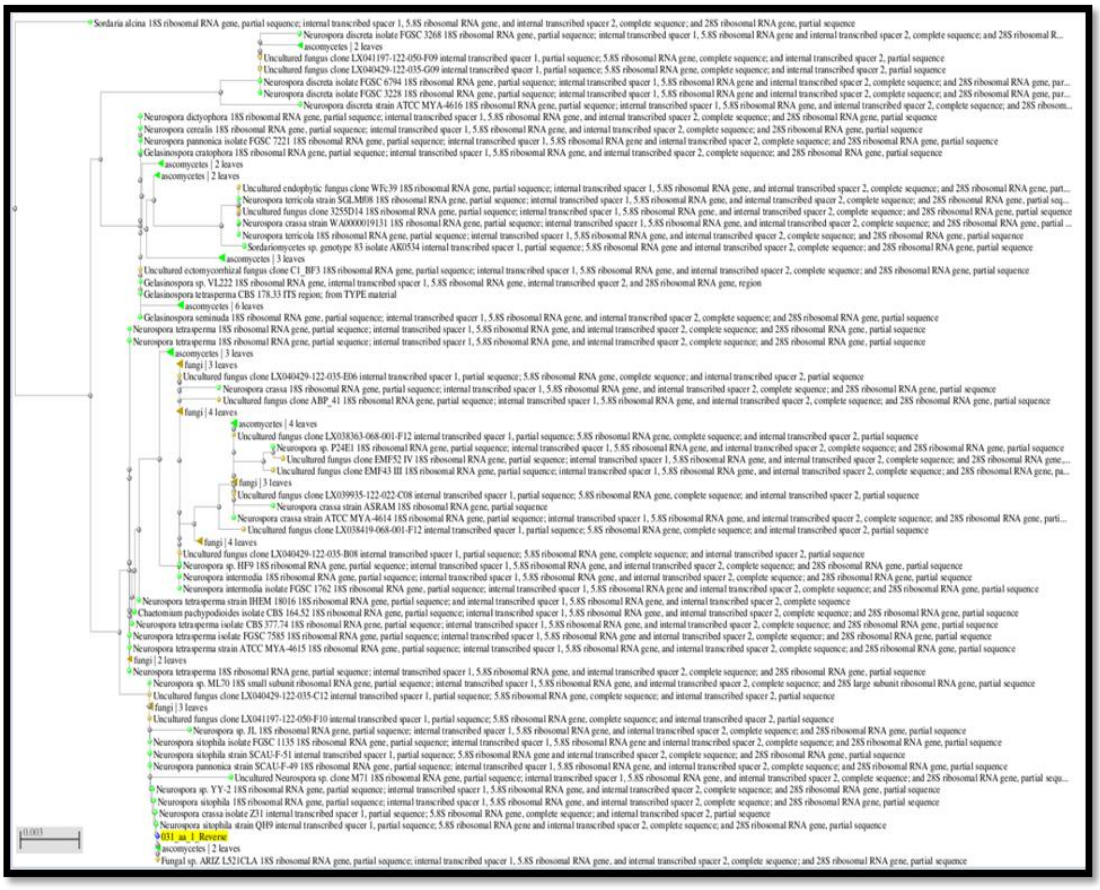
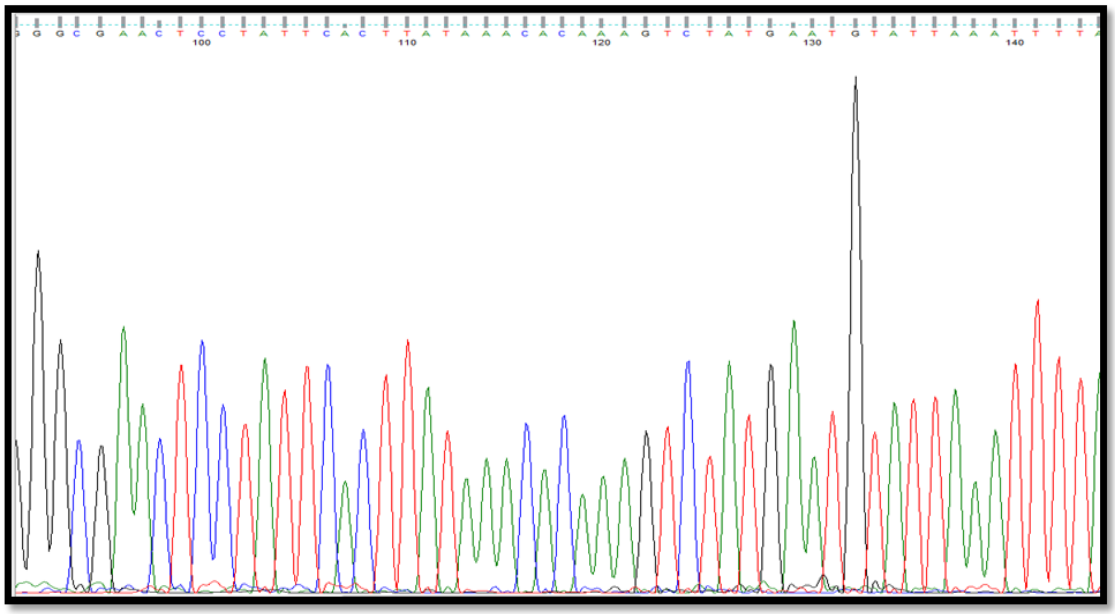


- ***Sporidiobolales sp***



Sporidiobolales sp. LM396 18S ribosomal RNA gene, partial sequence
Sequence ID: gb|EF060708.1|Length: 894Number of Matches: 1
Related Information
Range 1: 401 to 818GenBankGraphics

Score	Expect	Identities	Gaps	Strand
926 bits(1026)	0.0	513/513(100%)	0/513(0%)	Plus/Plus

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Query  1    GTGCATTTGTTTGGGATAGTAACTCTCGCAAGAGGGCGAACTCCTATTCACTTATAAACA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59   GTGCATTTGTTTGGGATAGTAACTCTCGCAAGAGGGCGAACTCCTATTCACTTATAAACA  118

Query  61   CAAAGTCTATGAATGTATTAAATTTTATAACAAAATAAAACTTTCAACAACGGATCTCTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  119  CAAAGTCTATGAATGTATTAAATTTTATAACAAAATAAAACTTTCAACAACGGATCTCTT  178

Query  121  GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  179  GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC  238

Query  181  AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCATGGTATTCCGTGGAGCATGCCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  239  AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCATGGTATTCCGTGGAGCATGCCT  298

Query  241  GTTTGAGTGTCATGAATACTTCAACCCTCCTCTTTCTTAATGATTGAAGAGGTGTTTGGT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  299  GTTTGAGTGTCATGAATACTTCAACCCTCCTCTTTCTTAATGATTGAAGAGGTGTTTGGT  358

Query  301  TTCTGAGCGCTGCTGGCCTTTAGGGTCTAGCTCGTTCGTAATGCATTAGCATCCGCAATC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  359  TTCTGAGCGCTGCTGGCCTTTAGGGTCTAGCTCGTTCGTAATGCATTAGCATCCGCAATC  418

Query  361  GAACTTCGGATTGACTTGGCGTAATAGACTATTCGCTGAGGAATTCTAGTCTTCGGACTA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  419  GAACTTCGGATTGACTTGGCGTAATAGACTATTCGCTGAGGAATTCTAGTCTTCGGACTA  478

Query  421  GAGCCGGGTTGGGTTAAAGGAAGCTTCTAATCAGAATGTCTACATTTTAAGATTAGATCT  480
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Sbjct  479  GAGCCGGGTTGGGTTAAAGGAAGCTTCTAATCAGAATGTCTACATTTTAAGATTAGATCT  538

Query  481  CAAATCAGGTAGGACTACCCGCTGAACTTAAGC  513
            |||||||||||||||||||||||||||||||||
Sbjct  539  CAAATCAGGTAGGACTACCCGCTGAACTTAAGC  571
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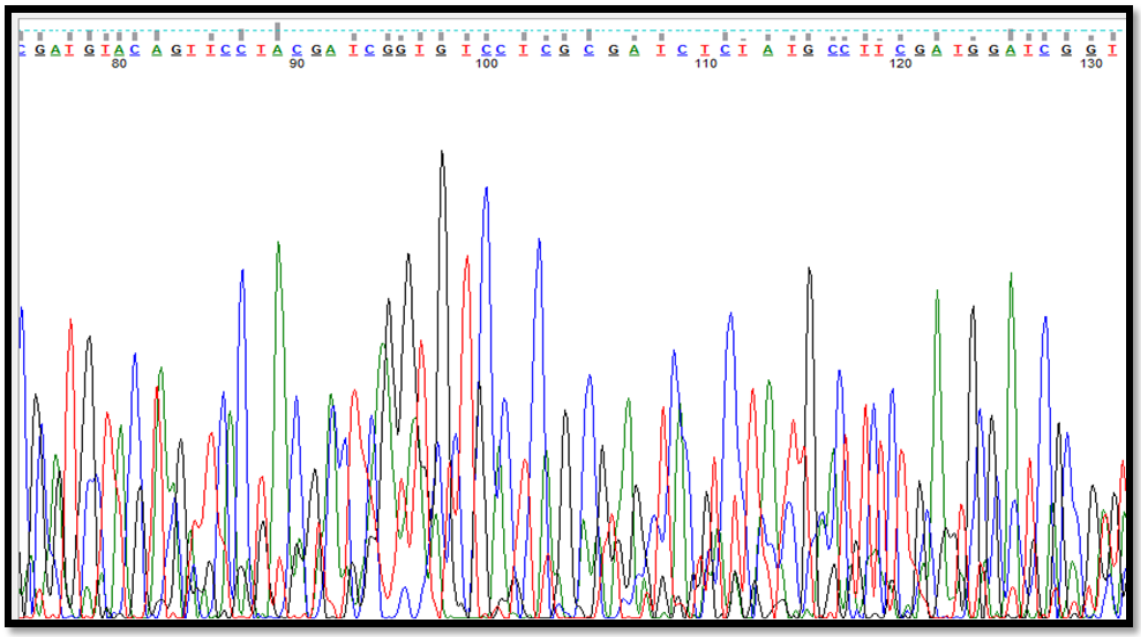
Rhodotorula mucilaginosa strain Y 17499 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured Sporidiobolales clone 11a 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Rhodotorula mucilaginosa strain CRUB 1100 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
Fungal endophyte sp. CEY 03 18S small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S large subunit ribosomal RNA gene, partial...
Rhodotorula mucilaginosa strain PYCC 4349 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
Rhodotorula mucilaginosa strain Y 17497 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
Rhodotorula mucilaginosa strain SQ 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
Rhodotorula mucilaginosa strain CRUB 1093 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
Rhodotorula sp. LH51 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 eukaryote clone NS4T_288 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 eukaryote clone NS4T_236 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 eukaryote clone NS4T_202 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
Rhodotorula mucilaginosa strain WP3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
basidiomycetes | 3 leaves
basidiomycetes | 3 leaves
basidiomycetes | 3 leaves
Uncultured fungus isolate FICUS 108 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
Rhodotorula mucilaginosa strain KDLYC24-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Rhodotorula mucilaginosa strain UOA/HCPF 10538 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
basidiomycetes | 3 leaves
Uncultured fungus isolate FICUS 079 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
Rhodotorula mucilaginosa isolate 66663 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
Rhodotorula mucilaginosa strain F1 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
Rhodotorula mucilaginosa culture-collection UOA/HCPF:GRC>12626P 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
Rhodotorula mucilaginosa genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, isolate Kw2715
Fungal sp. HZ30 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 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 26S rRNA, partial sequence, clone: Epi8-015
basidiomycetes | 2 leaves
Rhodotorula mucilaginosa isolate JD-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Rhodotorula sp. S-9801 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer, complete sequence; and 28S ribosomal RNA gene, partial sequence
Uncultured fungus ITS1, 5.8S rRNA gene and ITS2 region, clone f18
Uncultured fungus ITS1, 5.8S rRNA gene and ITS2 region, clone f2
Rhodotorula mucilaginosa strain SN27 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
Rhodotorula mucilaginosa strain UN131 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
Rhodotorula mucilaginosa strain ATCC 66034 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
Rhodotorula mucilaginosa strain CRUB 0526 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
Rhodotorula mucilaginosa strain CRUB 0034 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
Rhodotorula mucilaginosa strain Y 17485 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
Fungal endophyte sp. CEY 04 18S small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S large subunit ribosomal RNA gene, partial sequence
basidiomycetes | 5 leaves
Uncultured eukaryote clone NS31T_257 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 eukaryote clone NS31T_253 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
Rhodotorula mucilaginosa strain YD2 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
Rhodotorula mucilaginosa strain YM3 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
basidiomycetes | 6 leaves
Rhodotorula mucilaginosa strain Y5-8 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
basidiomycetes | 2 leaves
Rhodotorula mucilaginosa strain Y5-10 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
Rhodotorula mucilaginosa strain DY115-21-6-Y8 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
Rhodotorula mucilaginosa strain CBS 9070 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
Rhodotorula mucilaginosa strain K1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Rhodotorula mucilaginosa strain CRUB 1131 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
Rhodotorula mucilaginosa strain CRUB 1027 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
Rhodotorula mucilaginosa strain PTD3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Rhodotorula sp. PS4 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
Sporobolomyces sp. AL-V isolate AL-V-37 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 isolate FICUS 107 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
Rhodotorula mucilaginosa strain D31-2 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
Rhodotorula mucilaginosa strain AUMC 7248 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 | 2 leaves
Rhodotorula mucilaginosa strain AUMC 7778 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 isolate FICUS 104 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 Rhodotorula clone 873 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
Rhodotorula mucilaginosa strain UIMC35 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
Rhodotorula mucilaginosa strain YSC2 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 isolate FICUS 115 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
Rhodotorula mucilaginosa strain SC2 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
Antarctic fungal sp. GI948 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
basidiomycetes | 2 leaves
Antarctic fungal sp. GI947 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 | 4 leaves
Antarctic fungal sp. GI928 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
Antarctic fungal sp. GI926 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
Antarctic fungal sp. GI902 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
0.001
020_aa_2_Forward
basidiomycetes | 2 leaves

- ***Neurospora tetrasperm***



Neurospora tetrasperma strain XF35 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

Sequence ID: gb|JX136749.1|Length: 587Number of Matches: 1

Related Information

Range 1: 1 to 391GenBankGraphics

Score	Expect	Identities	Gaps	Strand
179 bits(198)	3e-41	279/391(71%)	6/391(1%)	Plus/Minus

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Query  114  AGCATGATGGATGACCTGACGGTCGACCCGAT-TGATCGCCACACTCCTCTTCAGTCCAT  172
            ||| |||||| |||  ||||| |||| | |   || |||||| | | ||    ||  ||
Sbjct  391  AGCTTGATGGTTGAAATGACGCTCGAACAGGCATGCTCGCCAGAATACTGGCGAGCGCAA  332

Query  173  TGTACGAACGATGATTCAATGATTCATTGCATTCTGGCATGCACTCTCCT-ATCCCATTT  231
            ||| ||   | | ||||| ||||||||  || ||||||   || |||   | || ||| |||||
Sbjct  331  TGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTT  272

Query  232  CGCTGCGCTCTTTATCGATCCTCGATCCTACAAATCCGTTGTTCTTC-TTTTCACTTATC  290
            ||||||| |||| ||||||  |   || || | | ||||||||||      |||| ||||||
Sbjct  271  CGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGACTTATT  212

Query  291  TCATCTCTTTTCCCTACACACTTCATCTATCCCCAGTTTATTTTCTGCATTCAGTTAGGT  350
            | |    ||   |  | | || | |  ||||   |||||| |||| ||| || |   ||
Sbjct  211  TAAAAGTTTACTCAGAGAGACATAAAATATCAAGAGTTTAGTTTCGGCACTCCGGCGGGC  152

Query  351  TTCCTTTCGTTATCGGGATACCCAAGGACCTGGGATTACCCTATGGACTTTCCTGACCGC  410
              |||  ||  | ||||| |||| |||| | ||||  |||| | || ||||||  ||||||
Sbjct  151  AGCCTCCCGCGAGCGGGAGACCCGAGGATCCGGGAGGACCCGAGGGCCTTTCCGGACCGC  92

Query  411  C-GAGCCCATGCCACCATACGGTAAACATTCCCGATGGTTTGTGGGAGTTTTGCAAATCT  469
            | | ||| | || ||| |||||  || |||| |||||||||||||||||||||||| |||
Sbjct  91   CAGCGCCGAGGCAACCGTACGGGTAAGATTCGCGATGGTTTGTGGGAGTTTTGCAACTCT  32

Query  470  G-AATGATCCCTCCGGA-GTTCACTTACGGA  498
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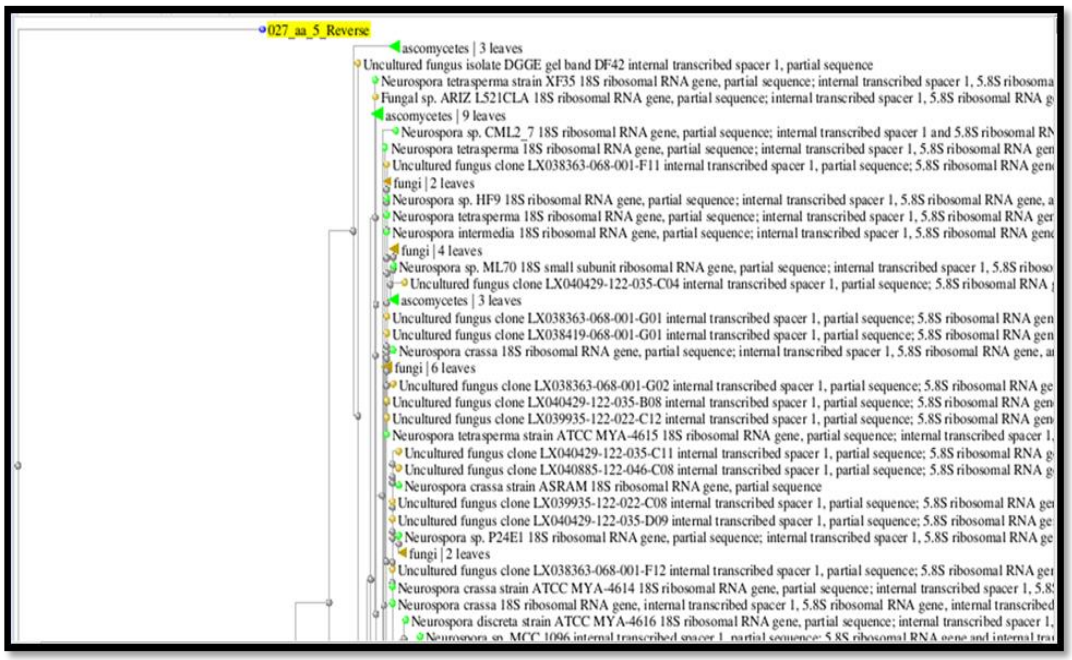
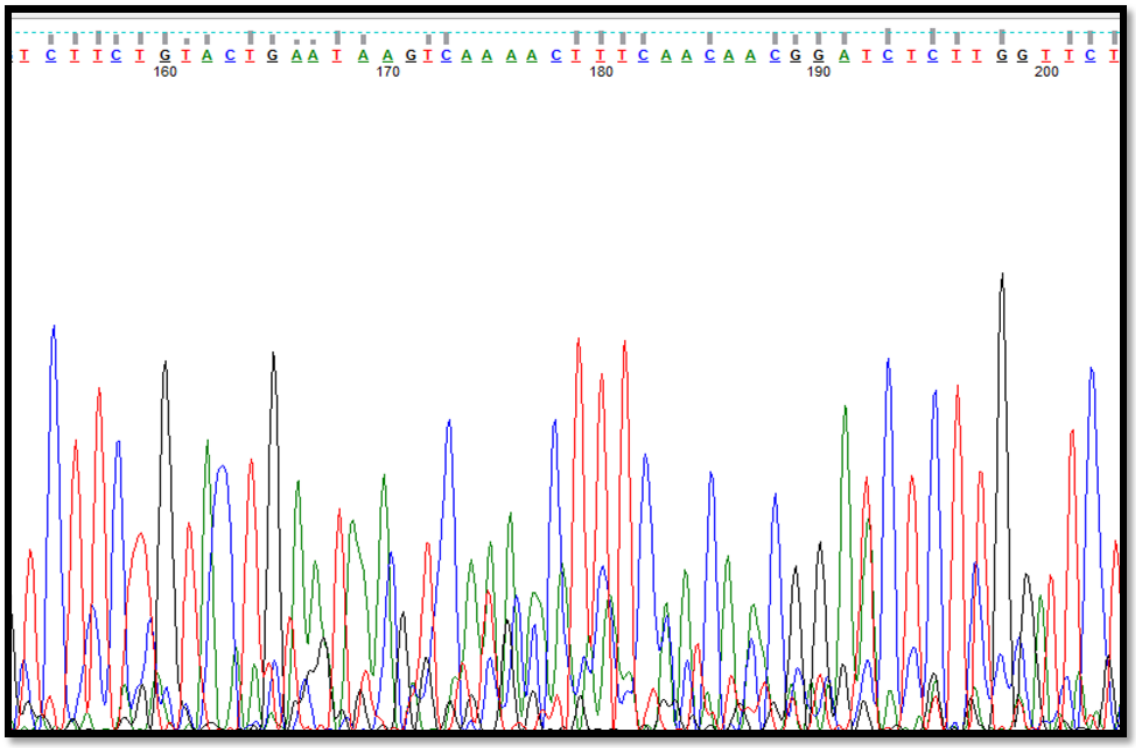


- ***Chaetomium megalocarpum***



Chaetomium megalocarpum strain CGMCC 3.3615 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

Sequence ID: gb|KC109743.1|Length: 1140Number of Matches: 1

Related Information

Range 1: 78 to 575GenBankGraphics

Score	Expect	Identities	Gaps	Strand
708 bits(784)	0.0	461/502(92%)	10/502(1%)	Plus/Plus

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Query  1    GTTGCTTCAGCGG-CGGCGCCAGGCTCTTTTAAA----GGGCGCCCCTGGGCCCCAAAGA  55
            ||||||||  ||||  |||||||  ||  |   |||        ||||||||||||||||   |
Sbjct  78   GTTGCTTCGGCGGGCGGCGCCGGGGT---TTACCCCCCGGGCGCCCCTGGGCCCCACCGC  134

Query  56   GGGCGCCCGCCCTATTT-ACCAAACTCTTGATAATTTATGGACACACTGAGTCTTCTGTA  114
            ||||||||||||   |  | ||||||||||||||||||||||||  | |  ||||||||||||||
Sbjct  135  GGGCGCCCGCCGGAGGTCACCAAACTCTTGATAATTTATGGCCTCTCTGAGTCTTCTGTA  194

Query  115  CTGAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAACAACGCA  174
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  195  CTGAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA  254

Query  175  GCGAAATGCGATAAGTAATHTHAATTHCAHAATTCAATGAATCATCGAATCTTTGAACGC  234
            ||||||||||||||||||  |  ||||  ||  ||||||  ||||||||||||||||||||||
Sbjct  255  GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC  314

Query  235  ACATTGCTCCCGCCAGTATTCTGGCGGGCATGCCTGTTCAAGCGTCATTTCAACCATCAA  294
            |||||||  ||||||||||||||||||||||||||||||||  ||||||||||||||||||||
Sbjct  315  ACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCATCAA  374

Query  295  GCCCCGGGCTTGTGTTGGGGACCTGCGGCTGCCGCAGGCCCTGAAAAGCAGTGGCGGGCT  354
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  375  GCCCCGGGCTTGTGTTGGGGACCTGCGGCTGCCGCAGGCCCTGAAAAGCAGTGGCGGGCT  434

Query  355  CGCTGTCACACCGAGCGTATTATTTTACTTCTCGCTCTGGGCGTGCTGCGGGTTCCGGCC  414
            ||||||||||||||||||  ||    |||  ||||||||||||||||||||||||||||||
Sbjct  435  CGCTGTCACACCGAGCGTAGTAGCATACATCTCGCTCTGGGCGTGCTGCGGGTTCCGGCC  494

Query  415  GTTAAACCACCTTTTAACCCAAGGTTGACCTCGGATCATGTAAGGAAAACCCGCTGAACT  474
            ||||||||||||||||||||||||||||||||||||||  ||  |||||  ||||||||||||
Sbjct  495  GTTAAACCACCTTTTAACCCAAGGTTGACCTCGGATCAGGT-AGGAAGACCCGCTGAACT  553

Query  475  TAAGCATATCAATAACCGGAGG  496
            ||||||||||||||  ||||||
Sbjct  554  TAAGCATATCAATAAGCGGAGG  575
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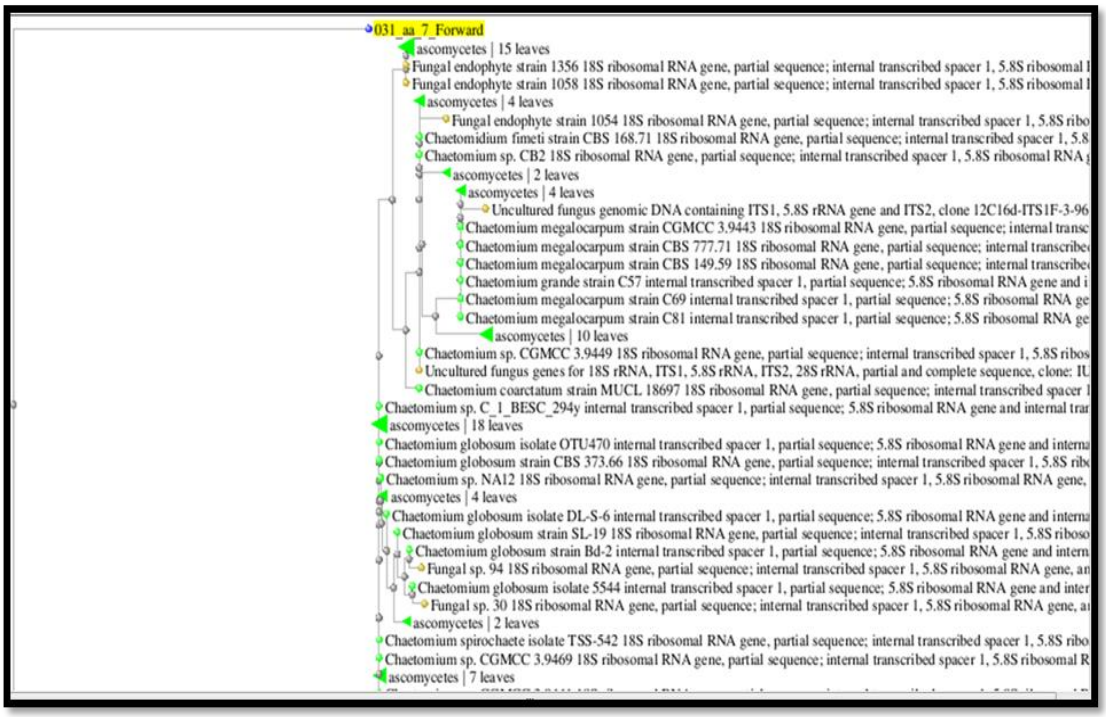
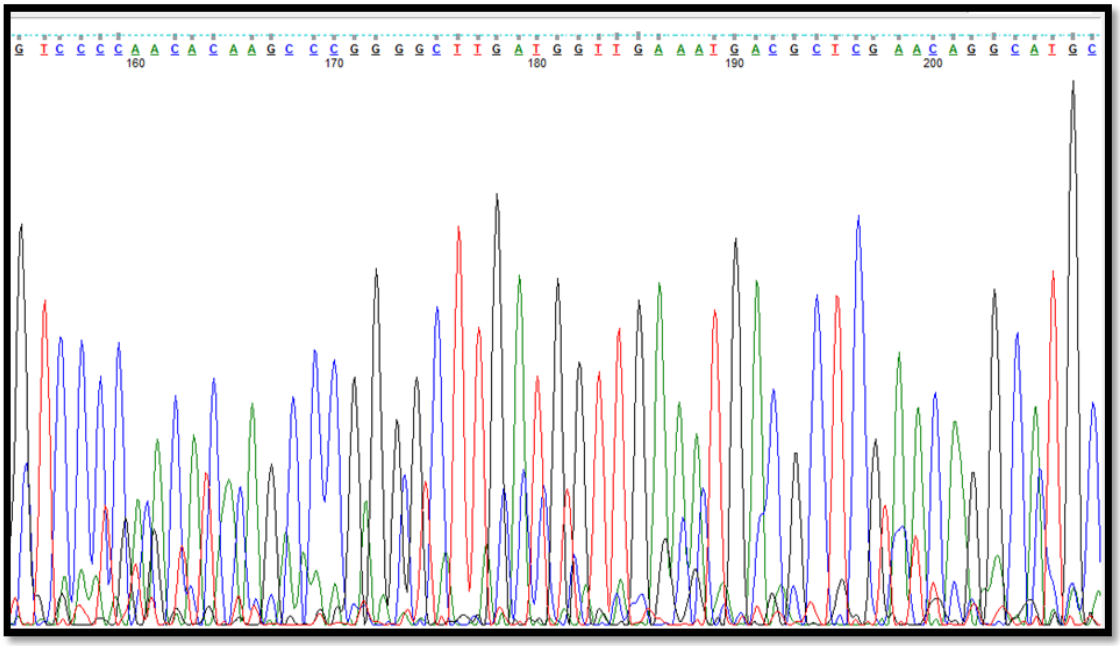


- ***Chaetomium globosum***



Chaetomium globosum isolate TNAU Cg16 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
Sequence ID: gb|JQ964802.1|Length: 750Number of Matches: 2

Score	Expect	Identities	Gaps	Strand
832 bits(922)	0.0	484/499(97%)	0/499(0%)	Plus/Minus

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Query  1    GGTTTACCGGCCCGACCCCGCACCCCGCCCAGAGCGAGATGTATGCTACTACGCTCGGTG  60
Sbjct  707  GGTTTAACGGCCGGAACCCGCAGCACGCCCAGAGCGAGATGTATGCTACTACGCTCGGTG  648

Query  61   TGACAGCGAGCCCGCCACTGCTTTTCCGGGCCTGCGGCAGCCGCAGGTCCCCAACACAAG  120
Sbjct  647  TGACAGCGAGCCCGCCACTGCTTTTCAGGGCCTGCGGCAGCCGCAGGTCCCCAACACAAG  588

Query  121  CCCGGGGCTTGATGGTTGAAATGACGCTCGAACAGGCATGCCCGCCCGACTACTGGCGGG  180
Sbjct  587  CCCGGGGCTTGATGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGG  528

Query  181  CGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCCATTCACATTACTTATCG  240
Sbjct  527  CGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCG  468

Query  241  CATTTCGCTGCCTTCTTCATCGATGCCAAAACCAAGAGATCCGTTGTTGAAAGTTTTGAC  300
Sbjct  467  CATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGAC  408

Query  301  TTATTCCGTACAGAACACTCACAGAGGCCATAAATTATCAAGAGTTTGGTGACCTccggc  360
Sbjct  407  TTATTCAGTACAGAAGACTCAGAGAGGCCATAAATTATCAAGAGTTTGGTGACCTCCGGC  348

Query  361  gggcgcccgcggtggggcccaggggcgcccgggggtaaacccccggcgcccgcccgccgAA  420
Sbjct  347  GGGCGCCCGCGGTGGGGCCCAGGGGCGCCCGGGGGGTAAACCCCGGCGCCGCCCGCCGAA  288

Query  421  GCATCGGTTTAGGTAACGTTCACAATGGTTTAGGGAGTTTTGCAACTCTGTAATGATCCC  480
Sbjct  287  GCAACGGTTTAGGTAACGTTCACAATGGTTTAGGGAGTTTTGCAACTCTGTAATGATCCC  228

Query  481  TCCGCAGGTTCACCTACGG  499
Sbjct  227  TCCGCAGGTTCACCTACGG  209
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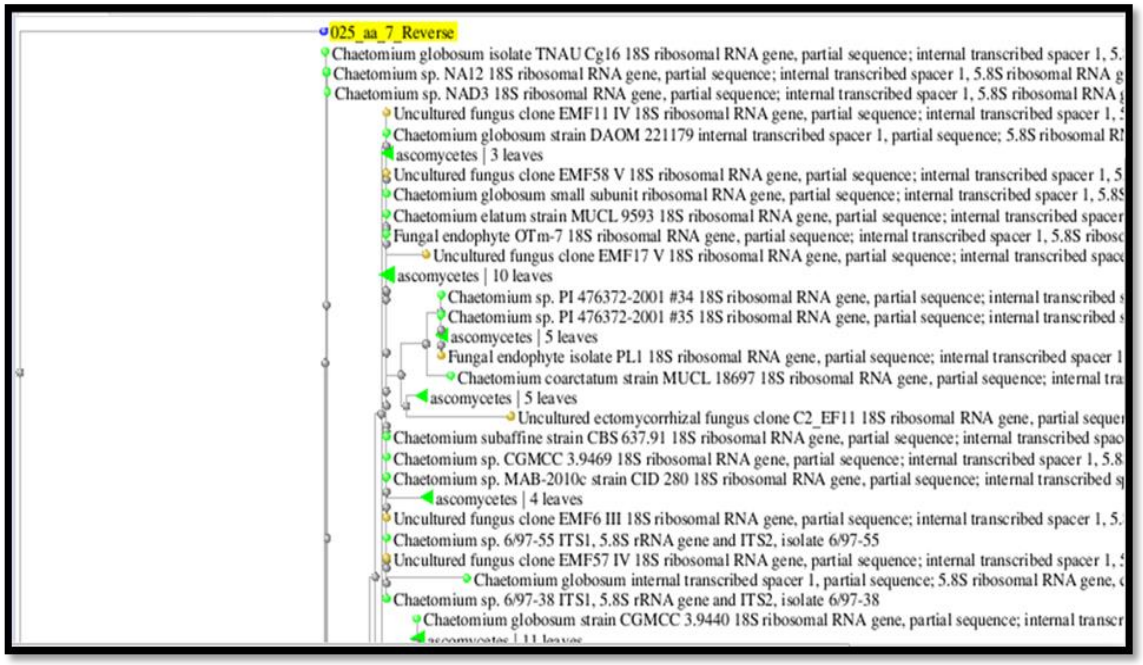
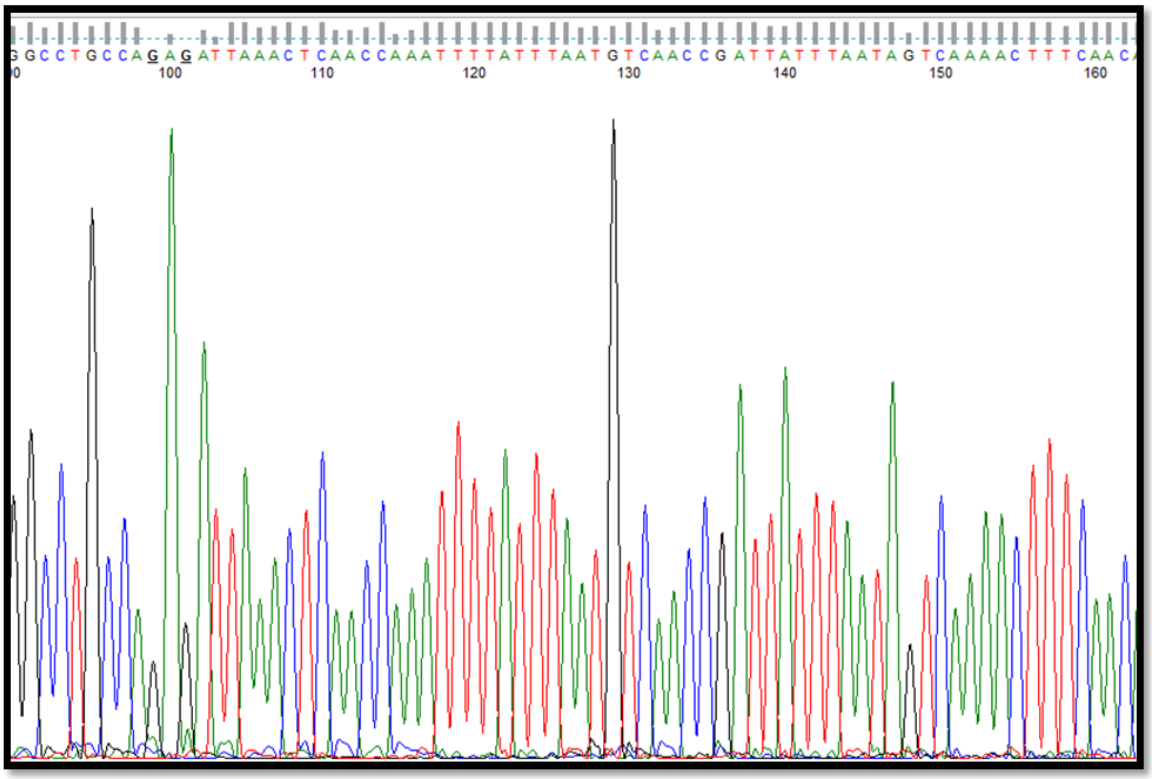


- ***Candida parapsilosis***



Candida parapsilosis isolate MRU1366 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

Sequence ID: gb|KM113999.1|Length: 472Number of Matches: 1

Related Information

Range 1: 39 to 472GenBankGraphics

Score	Expect	Identities	Gaps	Strand
783 bits(868)	0.0	434/434(100%)	0/434(0%)	Plus/Plus

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Query  1    GAAAACTTTGCTTTGGTAGGCCTTCTATATGGGGCCTGCCAGAGATTAAACTCAACCAAA  60
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Sbjct  39   GAAAACTTTGCTTTGGTAGGCCTTCTATATGGGGCCTGCCAGAGATTAAACTCAACCAAA  98

Query  61   TTTTATTTAATGTCAACCGATTATTTAATAGTCAAAACTTTCAACAACGGATCTCTTGGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  99   TTTTATTTAATGTCAACCGATTATTTAATAGTCAAAACTTTCAACAACGGATCTCTTGGT  158

Query  121  TCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATATTCGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  159  TCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATATTCGT  218

Query  181  GAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  219  GAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTT  278

Query  241  TGAGCGTCATTTCTCCCTCAAACCCTCGGGTTTGGTGTTGAGCGATACGCTGGGTTTGCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  279  TGAGCGTCATTTCTCCCTCAAACCCTCGGGTTTGGTGTTGAGCGATACGCTGGGTTTGCT  338

Query  301  TGAAAGAAAGGCGGAGTATAAACTAATGGATAGGTTTTTTCCACTCATTGGTACAAACTC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  339  TGAAAGAAAGGCGGAGTATAAACTAATGGATAGGTTTTTTCCACTCATTGGTACAAACTC  398

Query  361  CAAAACTTCTTCCAAATTCGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  399  CAAAACTTCTTCCAAATTCGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATA  458

Query  421  TCAATAAGCGGAGG  434
            ||||||||||||||
Sbjct  459  TCAATAAGCGGAGG  472
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ascomycetes | 75 leaves

Uncultured fungus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, clone: DSF_33_BO32

Candida albicans strain wxj32-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, c...

Candida parapsilosis strain A005 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

Candida parapsilosis strain UOA/HCPF 1B1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed...

Candida parapsilosis strain EN22 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

Candida parapsilosis strain 157 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S r...

Candida parapsilosis isolate ZA039 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...

Candida parapsilosis isolate ZA033 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...

Candida parapsilosis isolate ZA031 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...

Candida parapsilosis isolate ZA012 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...

Candida parapsilosis isolate ZA007 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...

Candida parapsilosis strain UM7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, c...

Candida parapsilosis strain SJ26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, c...

Candida parapsilosis strain NRRL Y-12969 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed ...

Candida parapsilosis 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), isolate Kw113-98

Candida parapsilosis strain ATCC 90018 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed sp...

Candida parapsilosis strain L6492 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

Candida parapsilosis strain L6550 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

Candida parapsilosis strain L8096 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

Candida parapsilosis strain L7936 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

Candida parapsilosis strain L5974 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

Candida parapsilosis strain L8124 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

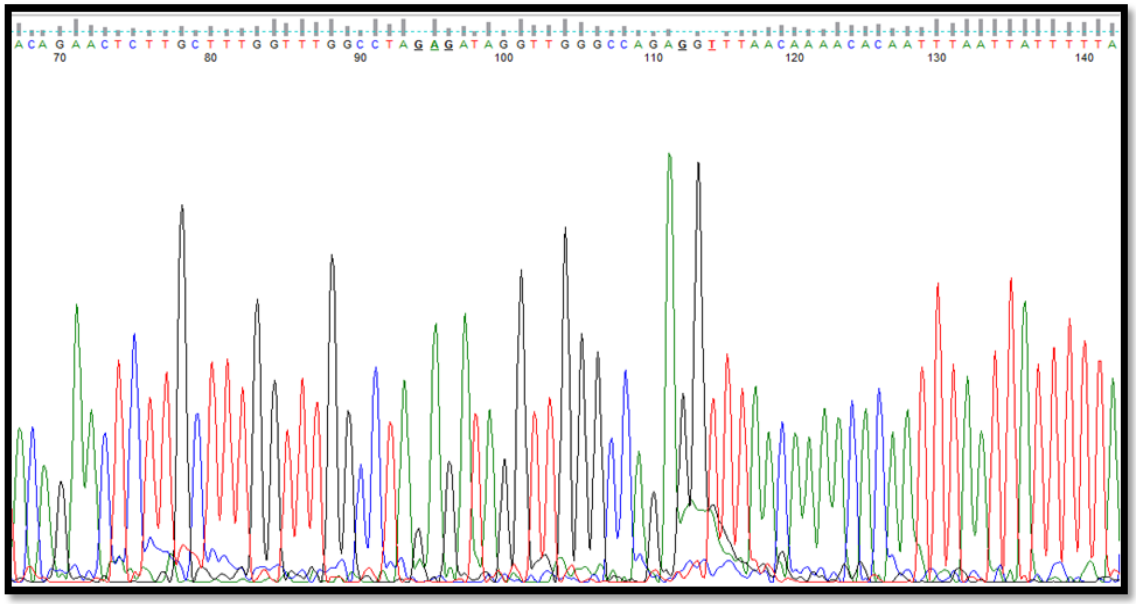
Candida parapsilosis strain L7929 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, ...

Candida parapsilosis strain L8035A 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...

Candida parapsilosis strain L8910A 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,...

028_aa_10_Forward

- ***Meyerozyma guilliermondii***



Meyerozyma guilliermondii strain JY 41 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
Sequence ID: gb|KM014576.1|Length: 707Number of Matches: 1
Related Information
Range 1: 59 to 606GenBank

Score	Expect	Identities	Gaps	Strand
989 bits(1096)	0.0	548/548(100%)	0/548(0%)	Plus/Plus

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Sbjct  59   GGCGAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAG  118

Query  61   AGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAA  120
Sbjct  119  AGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAA  178

Query  121  TTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  180
Sbjct  179  TTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  238

Query  181  ACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTG  240
Sbjct  239  ACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTG  298

Query  241  AACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTC  300
Sbjct  299  AACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTC  358

Query  301  TCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAA  360
Sbjct  359  TCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAA  418

Query  361  AGTATTGGCATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCC  420
Sbjct  419  AGTATTGGCATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCC  478

Query  421  AACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACC  480
Sbjct  479  AACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACC  538

Query  481  AAACAAGTTTGACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGC  540
Sbjct  539  AAACAAGTTTGACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGC  598

Query  541  GGAGGAAA  548
Sbjct  599  GGAGGAAA  606
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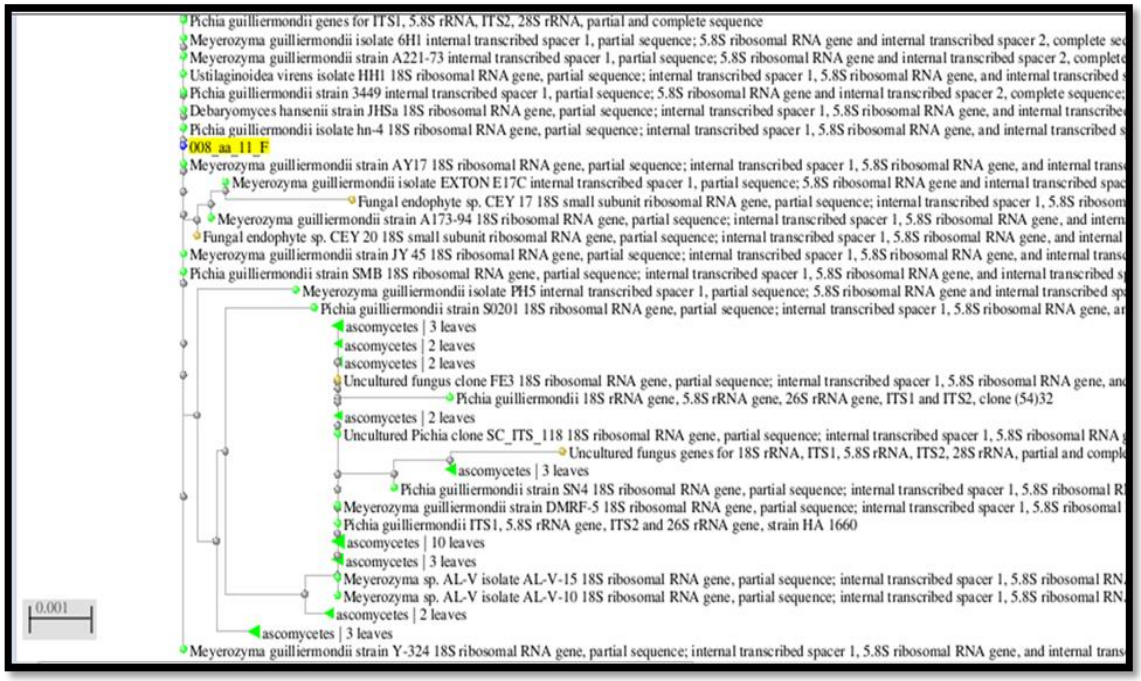
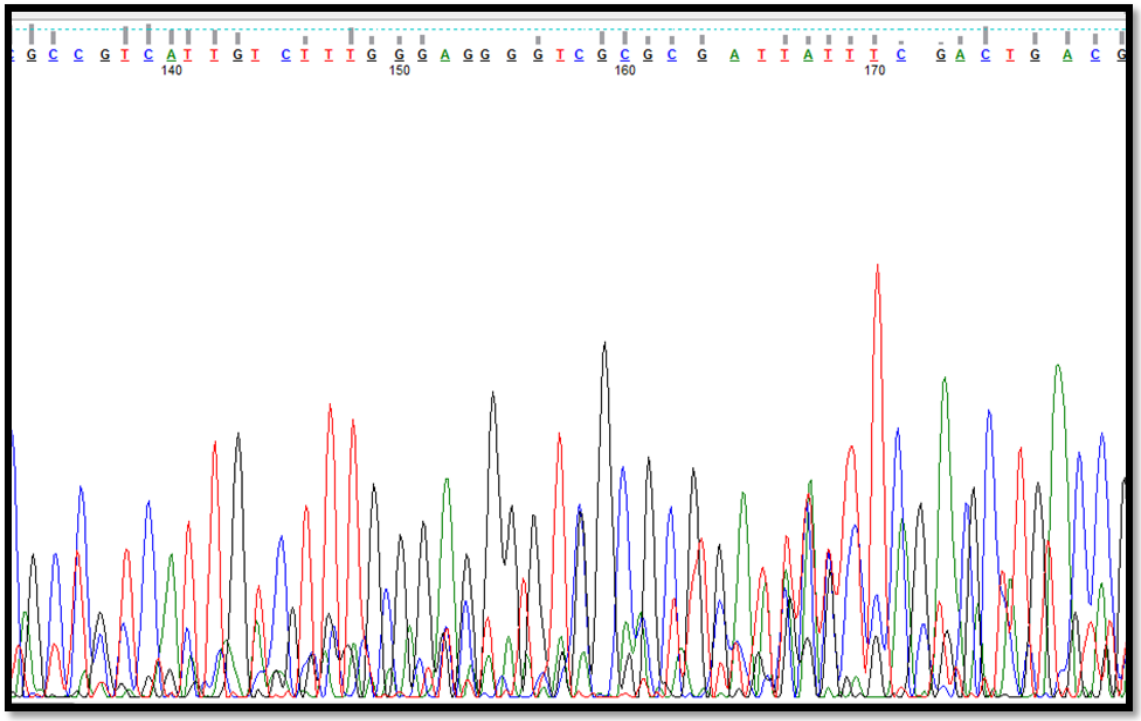


- ***Exophiala dermatitidis***



Exophiala dermatitidis isolate 95B 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
Sequence ID: gb|KJ740171.1|Length: 592Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
989 bits(1096)	0.0	548/548(100%)	0/548(0%)	Plus/Plus

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Query  1    GGCGAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAG  60
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Sbjct  59   GGCGAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAG  118

Query  61   AGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  119  AGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAA  178

Query  121  TTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  179  TTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  238

Query  181  ACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  239  ACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTG  298

Query  241  AACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  299  AACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTC  358

Query  301  TCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAA  360
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Sbjct  359  TCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAA  418

Query  361  AGTATTGGCATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  419  AGTATTGGCATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCC  478

Query  421  AACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  479  AACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACC  538

Query  481  AAACAAGTTTGACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539  AAACAAGTTTGACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGC  598

Query  541  GGAGGAAA  548
            ||||||||
Sbjct  599  GGAGGAAA  606
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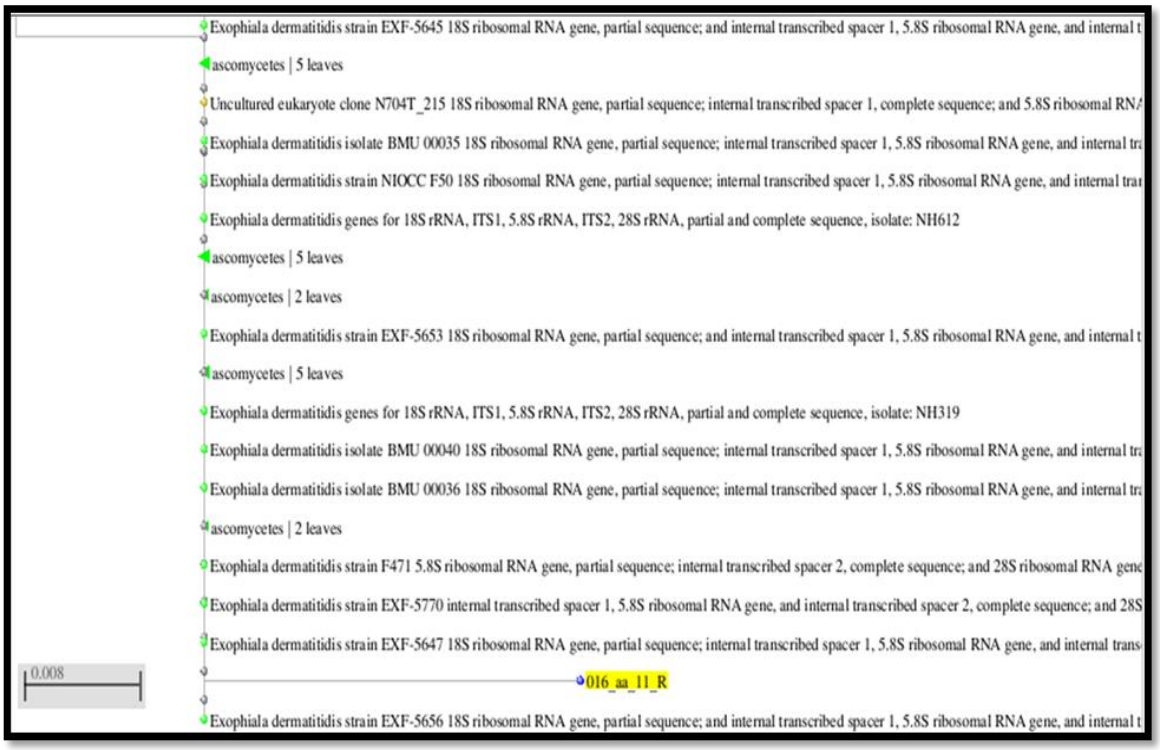
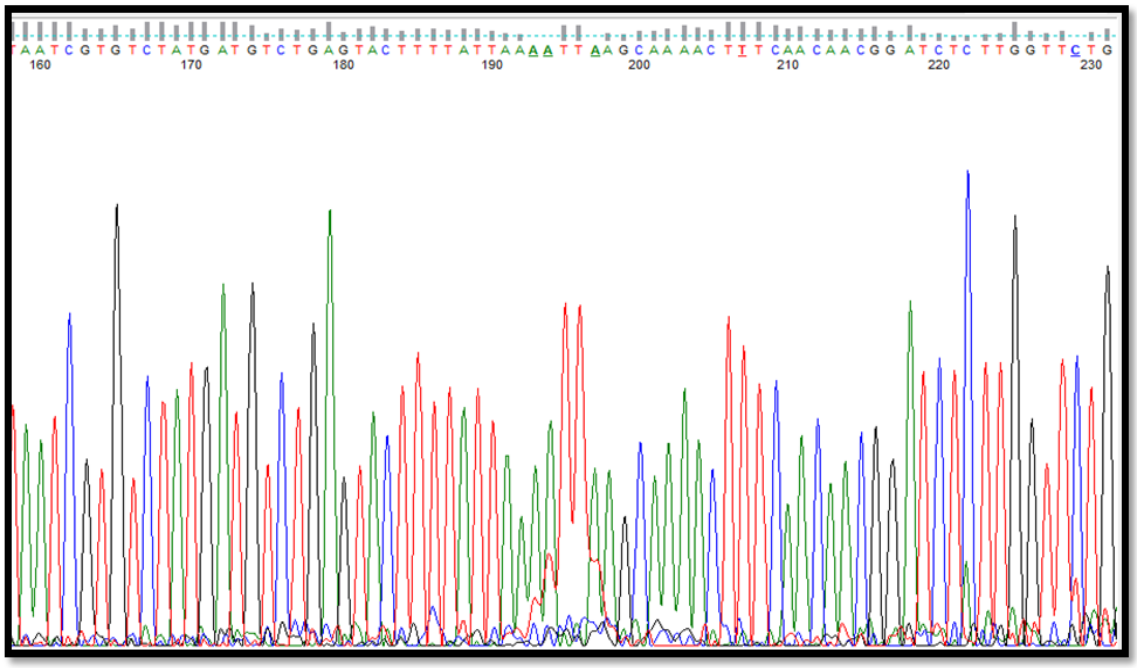


- ***Exophiala phaeomuriformis***



Exophiala phaeomuriformis strain CBS 137224 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

Sequence ID: gb|KJ522802.1|Length: 690Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
517 bits(572)	6e-143	317/335(95%)	3/335(0%)	Plus/Plus

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Query  1    GAACCCTTGTTGCTTCGG-GGGCCCTT--TTAGGGGGCCGCCGGAGGACCGACTCCAGGT  57
            |||||||||||||||||| |||||| |  |||   ||||||||||||||||||||||||||
Sbjct  49   GAACCCTTGTTGCTTCGGCGGGCCCGTCGTTATACGGCCGCCGGAGGACCGACTCCAGGT  108

Query  58   CCTCTGCTTTTCGCCCGCCGGTAGCCAAAATCACCAAACTCTTGAATAATCGTGTCTATG  117
            ||||||     ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  109  CCTCTGGCCCGCGCCCGCCGGTAGCCAAAATCACCAAACTCTTGAATAATCGTGTCTATG  168

Query  118  ATGTCTGAGTACTTTTATTAAAATTAAGCAAAACTTTCAACAACGGATCTCTTGGTTCTG  177
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  169  ATGTCTGAGTACTTTTATTAAAATTAAGCAAAACTTTCAACAACGGATCTCTTGGTTCTG  228

Query  178  GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGCGAATTGCAGAATTCCAGTGAG  237
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  229  GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGCGAATTGCAGAATTCCAGTGAG  288

Query  238  TCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTCGA  297
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  289  TCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTCGA  348

Query  298  GCGCCATCATccccccccaaagcccccGGCTTGGT  332
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Sbjct  349  GCGTCATTATCACCCCTCAAGCCCTCCGGCTTGGT  383
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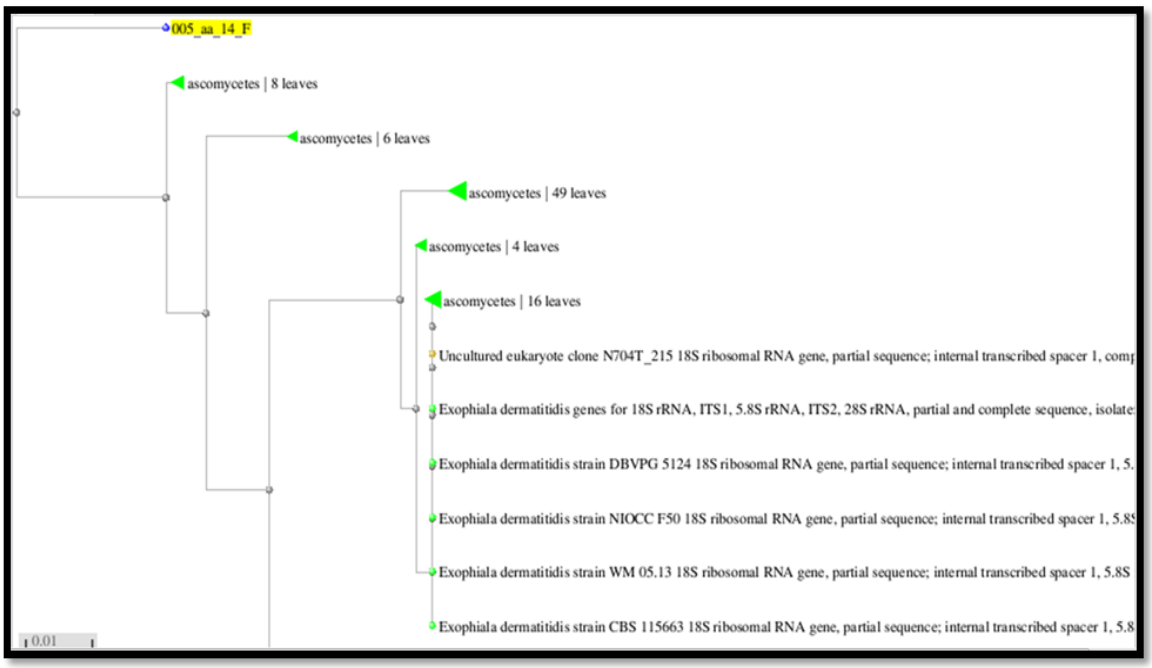
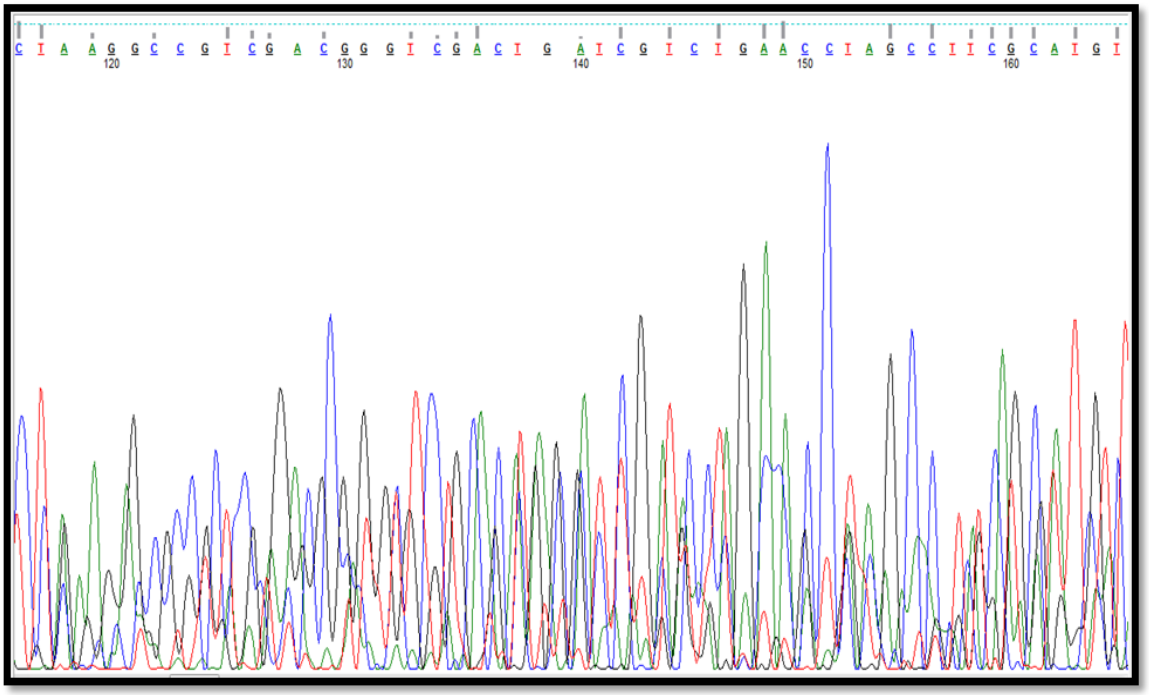

005_aa_14_F
ascomycetes | 8 leaves
ascomycetes | 6 leaves
ascomycetes | 49 leaves
ascomycetes | 4 leaves
ascomycetes | 16 leaves
Uncultured eukaryote clone N704T_215 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, comp
Exophiala dermatitidis genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate
Exophiala dermatitidis strain DBVPG 5124 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.
Exophiala dermatitidis strain NIOCC F50 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8
Exophiala dermatitidis strain WM 05.13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S
Exophiala dermatitidis strain CBS 115663 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8
0.01

- **Fungal sp**



Fungal sp. US14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: gb|JN802258.1|Length: 538Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
69.8 bits(76)	2e-08	109/152(72%)	3/152(1%)	Plus/Minus

```
Query  135  CGGTCGCA-TGTGCATTCATAA-TTAAATGATCTGCTGCATTCTGCAATTCGCA-AACAT  191
            ||| |||| ||||| |||| || || ||||||   ||| ||||||||||| ||  || |
Sbjct  302  CGGGCGCAATGTGCGTTCAAAAATTCAATGATTCACTGAATTCTGCAATTCACATTACTT  243

Query  192  AGAGCCTTTTGCTGTCTGCTTTTTCCATTACATAACCAATTGAACTGCTGTTGACATTTA  251
            |  || ||| ||||  | |||  || ||  || ||||||  || | | |||||| | ||
Sbjct  242  ATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTT  183

Query  252  TGAACCATCTGCTTTAGCGCGTGCTACTCAGA  283
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Sbjct  182  TGATTCATTTGTTTTTGCTTGTGCAACTCAGA  151
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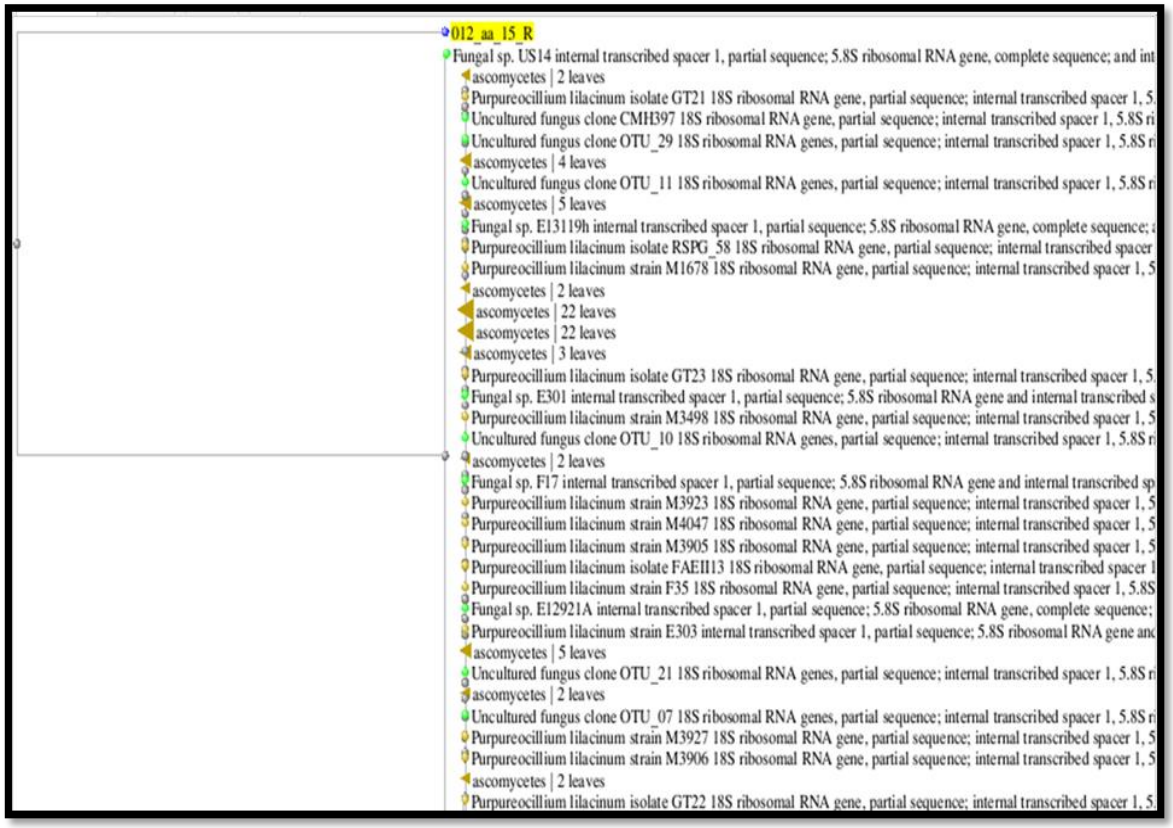
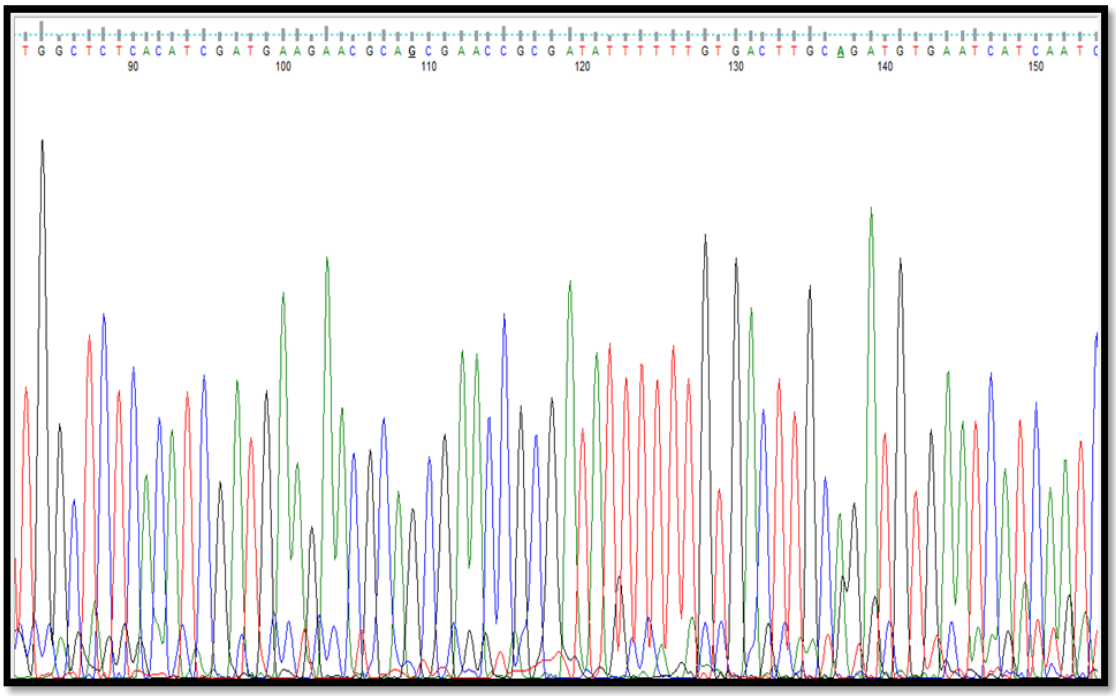


- ***Yarrowia lipolytica***



Yarrowia lipolytica strain M3 26S ribosomal RNA gene, partial sequence
Sequence ID: gb|KF851353.1|Length: 320Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
509 bits(564)	7e-141	286/289(99%)	0/289(0%)	Plus/Plus

```
Query  1    GCGTCATTTTATCTCAATTATAACTATCAACAACGGATCTCTTGGCTCTCACATCGATGA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  30   GCGTCATTTTATCTCAATTATAACTATCAACAACGGATCTCTTGGCTCTCACATCGATGA  89

Query  61   AGAACGCAGCGAACCGCGATATTTTTTGTGACTTGCAGATGTGAATCATCAATCTTTGAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  90   AGAACGCAGCGAACCGCGATATTTTTTGTGACTTGCAGATGTGAATCATCAATCTTTGAA  149

Query  121  CGCACATTGCGCGGTATGGCATTCCGTACCGCACGGATGGAGGAGCGTGTTCCCTCTGGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150  CGCACATTGCGCGGTATGGCATTCCGTACCGCACGGATGGAGGAGCGTGTTCCCTCTGGG  209

Query  181  ATCGCATTGCTTTNTTGAAATGGAttttttttAAACTCTCAATTATTACGTCATTTCACCT  240
            |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  210  ATCGCATTGCTTTCTTGAAATGGATTTTTTTTAAACTCTCAATTATTACGTCATTTCACCT  269

Query  241  CCTTCATCCAAGATTACCCGCTGAACTTAAGCATATCAAAAAGCGGAGG  289
            |||||||||||||||||||||||||||||||||||| || ||||||
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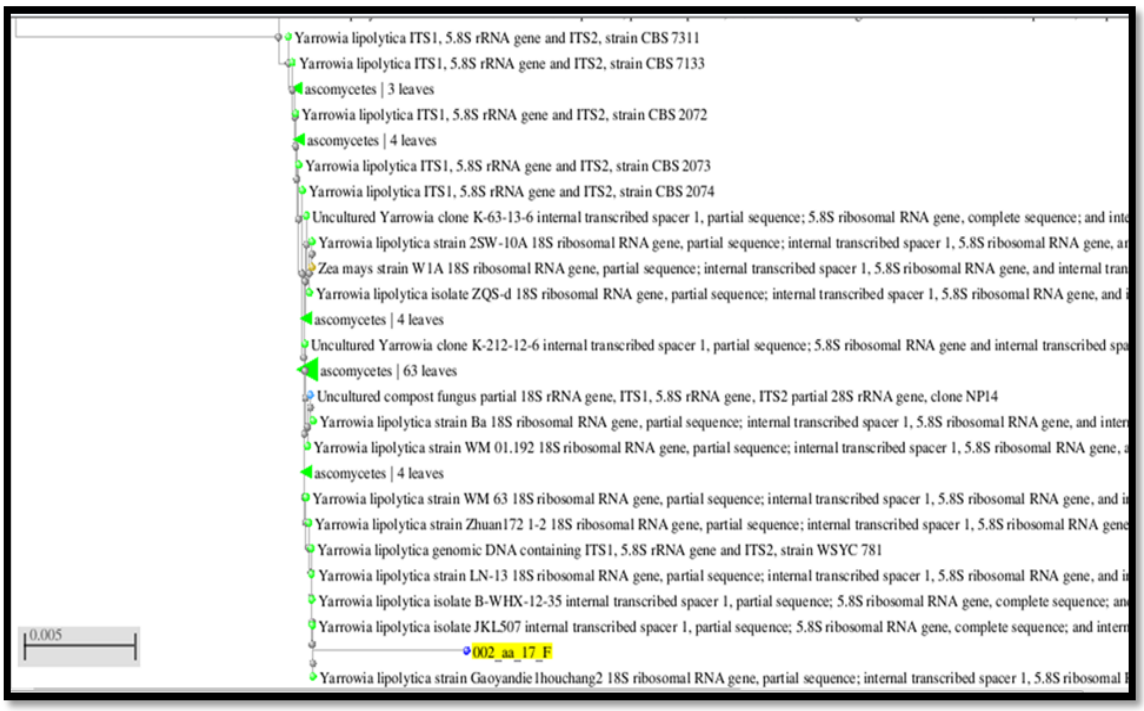
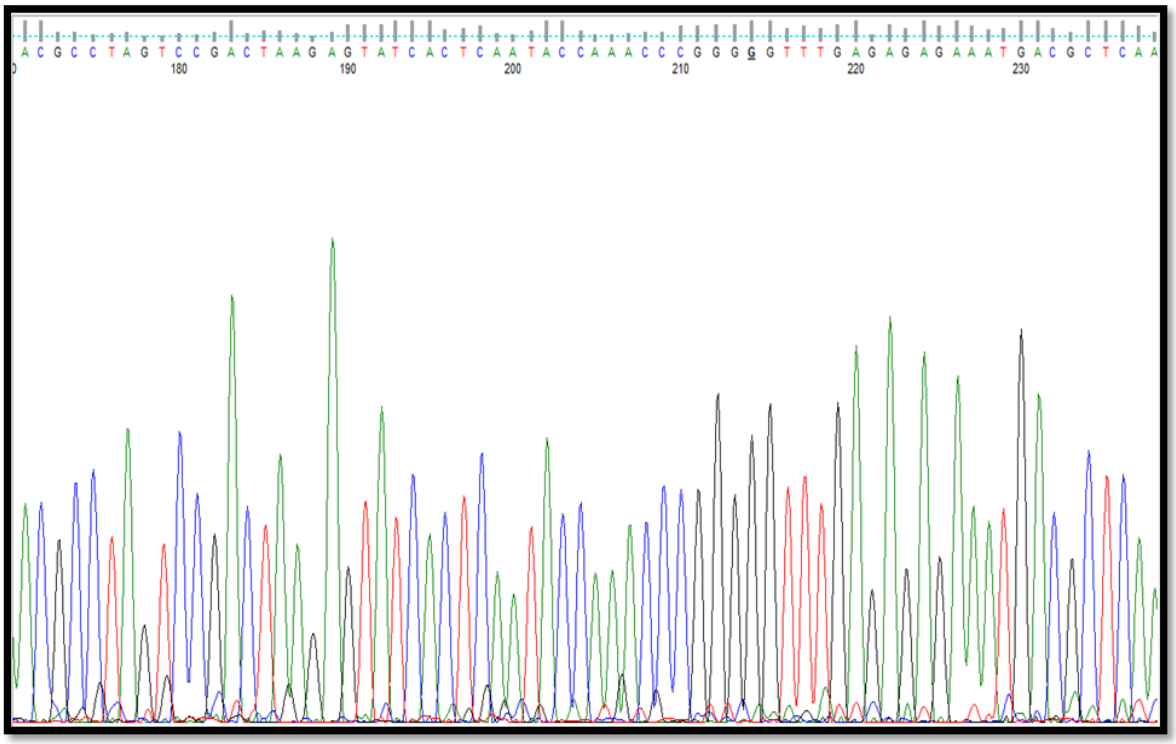


- ***Pichia guilliermondii***



Pichia guilliermondii isolate JHSd 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
Sequence ID: gb|DQ663478.1|Length: 617Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
960 bits(1064)	0.0	532/532(100%)	0/532(0%)	Plus/Minus

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Query  1    GGCCGGGCCAACAATACCAGAAATATCCCGCCACACCATTCAACGAGTTGGATAAACCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  540  GGCCGGGCCAACAATACCAGAAATATCCCGCCACACCATTCAACGAGTTGGATAAACCTA  481

Query  61   ATACATTGAGAGGTCGACAGCACTATCTAGTACTACCCATGCCAATACTTTTCAAGCAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  480  ATACATTGAGAGGTCGACAGCACTATCTAGTACTACCCATGCCAATACTTTTCAAGCAAA  421

Query  121  CGCCTAGTCCGACTAAGAGTATCACTCAATACCAAACCCGGGGGGTTTGAGAGAGAAATGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  420  CGCCTAGTCCGACTAAGAGTATCACTCAATACCAAACCCGGGGGGTTTGAGAGAGAAATGA  361

Query  181  CGCTCAAACAGGCATGCCCTCTGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  360  CGCTCAAACAGGCATGCCCTCTGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGA  301

Query  241  TGATTCACGAAAATCTGCAATTCATATTACTTATCGCATTTCGCTGCGTTCTTCATCGAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  300  TGATTCACGAAAATCTGCAATTCATATTACTTATCGCATTTCGCTGCGTTCTTCATCGAT  241

Query  301  GCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGAAGATTAATTCAAAATTTGACTAACT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  240  GCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGAAGATTAATTCAAAATTTGACTAACT  181

Query  361  GTAAAAATAATTAAATTGTGTTTTGTTAAACCTCTGGCCCAACCTATCTCTAGGCCAAAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  180  GTAAAAATAATTAAATTGTGTTTTGTTAAACCTCTGGCCCAACCTATCTCTAGGCCAAAC  121

Query  421  CAAAGCAAGAGTTCTGTATCAAAAAGACACTGTGTGTAAGGTTTTTCGCCGCGCAGTTAA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  120  CAAAGCAAGAGTTCTGTATCAAAAAGACACTGTGTGTAAGGTTTTTCGCCGCGCAGTTAA  61

Query  481  GCGCTGGCAAAAGAATACTGTAATGATCCTTCCGCAGGTTCACCTACGGAAG  532
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  60   GCGCTGGCAAAAGAATACTGTAATGATCCTTCCGCAGGTTCACCTACGGAAG  9
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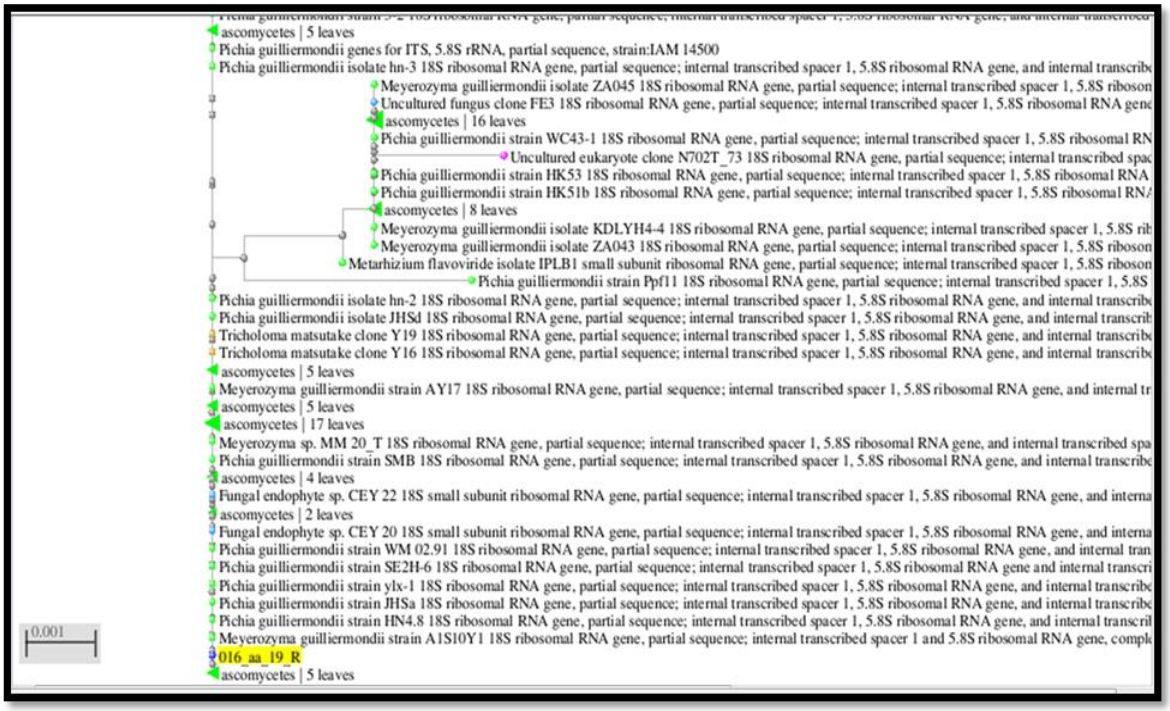
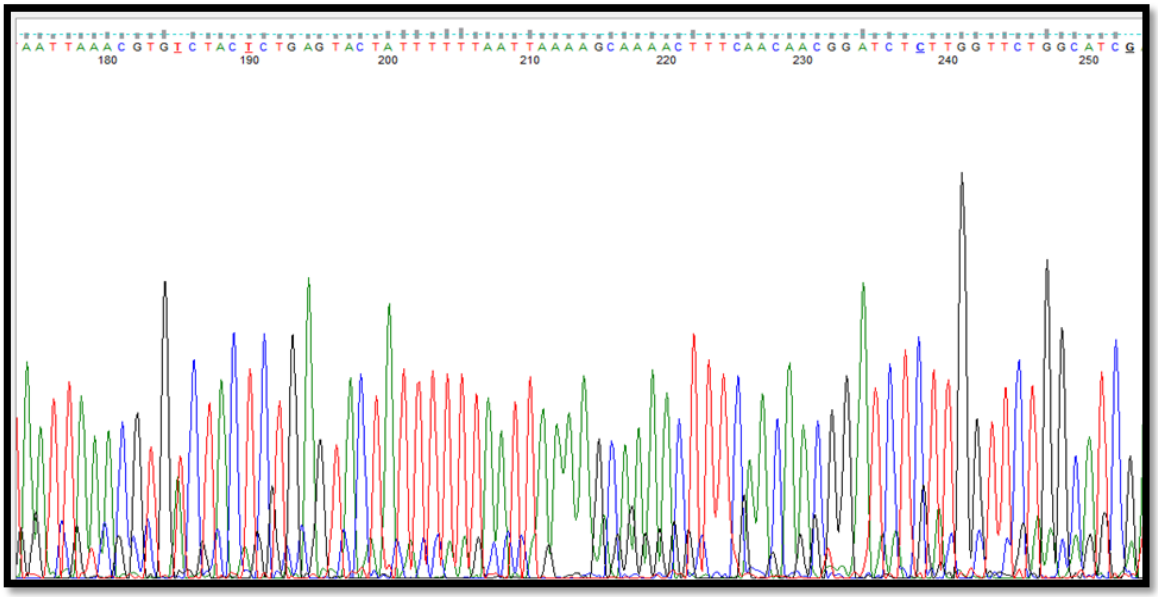


- ***Exophiala pisciphila***



Exophiala pisciphila CBS 464.81 18S ribosomal RNA partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2 complete sequence and 28S ribosomal RNA partial sequence

Sequence ID: gb|AF050273.1|AF050273 Length: 1500 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
966 bits(1070)	0.0	551/560(98%)	2/560(0%)	Plus/Plus

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Query  30   CGGAGAGCCCTTTTCTCCCTCTCCgggggggCAGACCGCCGGGGGGNCTTCAAACCGTCC  89
            ||| |||||| | ||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  73   CGGCGAGCCCGTCTCTCCCTCTCCGGGGGGGGCAGACCGCCGGGGGGCCTTCAAACCGTCC  132

Query  90   TCTGGCCCGCGCTCGTCGGTGGCCAACCTTTACAAATTCTTAATTAAACGTGTCTACTCT  149
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  133  TCTGGCCCGCGCTCGTCGGTGGCCAACCTTTACAAATTCTTAATTAAACGTGTCTACTCT  192

Query  150  GAGTACTATTTTTTAATTAAAAGCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC  209
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  193  GAGTACTATTTTTTAATTAAAAGCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC  252

Query  210  GATGAAGAACGCAGCGAAATGCGATAAGTAATGCGAATTGCAGAATTCCAGTGAGTCATC  269
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  253  GATGAAGAACGCAGCGAAATGCGATAAGTAATGCGAATTGCAGAATTCCAGTGAGTCATC  312

Query  270  GAATCTTTGAACGCACATTGCGCCCTTTGGCATTCCGAAGGGCATGCCTGTTCGAGCGTC  329
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  313  GAATCTTTGAACGCACATTGCGCCCTTTGGCATTCCGAAGGGCATGCCTGTTCGAGCGTC  372

Query  330  ATTATCACCTCTCAAGCCCCCCTTTTCTGGGCTTGGTGTTGGACGGCCTGGCGTCGGCGA  389
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  373  ATTATCACCTCTCAAGCCCCCCTTTTCTGGGCTTGGTGTTGGACGGCCTGGCGTCGGCGA  432

Query  390  CGACCCCACCTCCCAAAGACAATGACGGCGGNTTCGTGAGACCCCCGGTACACTGAGTTC  449
            |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  433  CGACCCCACCTCCCAAAGACAATGACGGCGGCTTCGTGAGACCCCCGGTACACTGAGTTC  492

Query  450  CTCACCGAACACGTACTGGATCAAGGGTAGACGGAGCCCCGGTCNACCTCCCCTCACAGG  509
            |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  493  CTCACCGAACACGTACTGGATCAAGGGTAGACGGAGCCCCGGTCGACCTCCCCTCACAGG  552

Query  510  GAGACACTTTTTTACAANGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA  569
            |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  553  GAGACACTTTTTTACAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA  612

Query  570  TATC-ATAAGCGGGAGGAAA  588
            |||| |||||| ||||||||
Sbjct  613  TATCAATAAGC-GGAGGAAA  631
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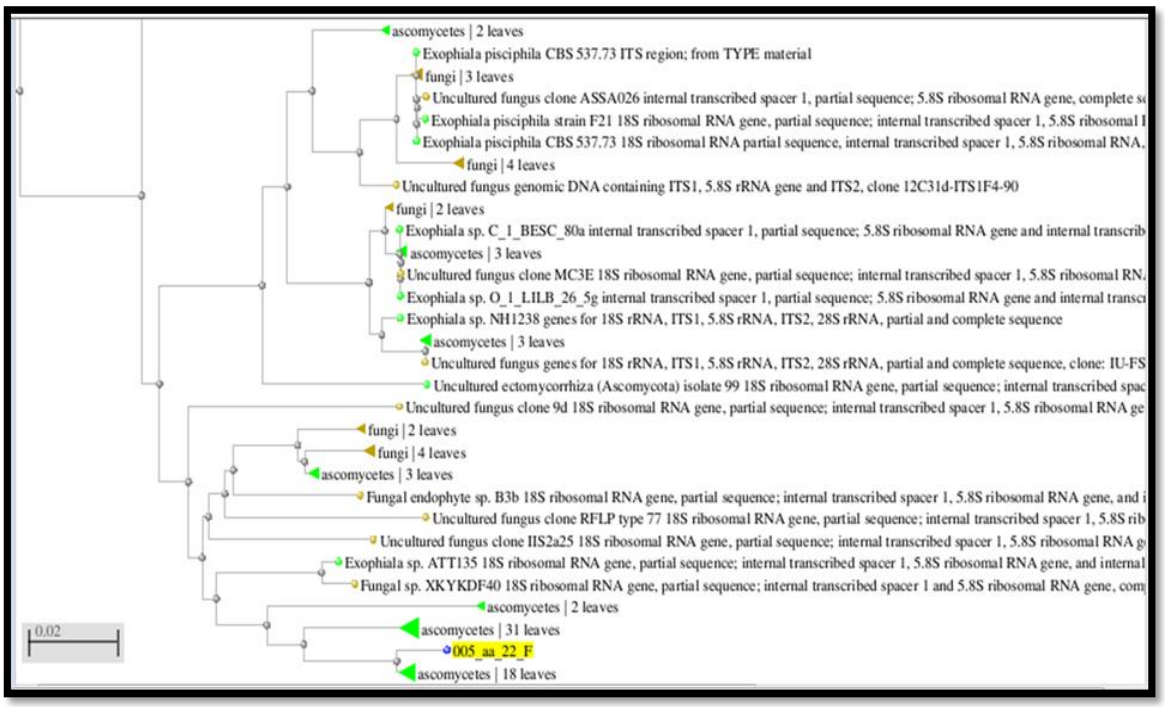
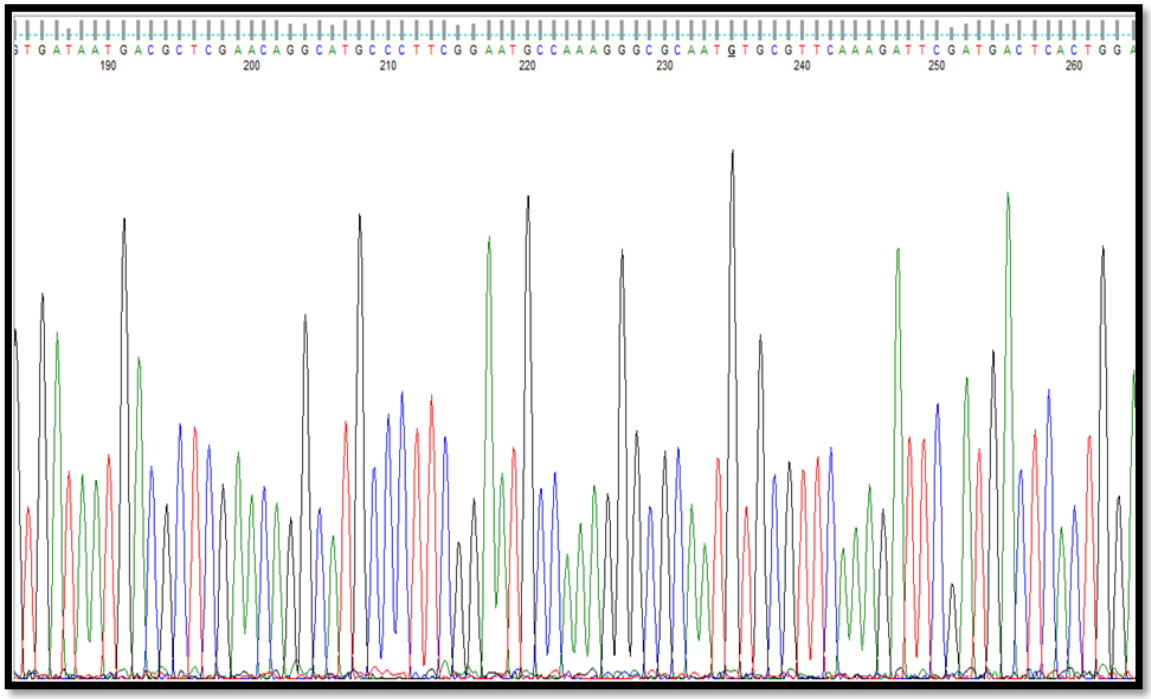


- ***Exophiala lecanii-corni***



Exophiala lecanii-corni strain CCFEE 5987 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

Sequence ID: gb|JX681040.1|Length: 605Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
951 bits(1054)	0.0	527/527(100%)	0/527(0%)	Plus/Minus

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Query  1    GATCCAGTACGTGTTCGGTGAGGAACTCAGTGTACCGGGGGTCTCACGAAGCCGCCGTCA  60
Sbjct  527  GATCCAGTACGTGTTCGGTGAGGAACTCAGTGTACCGGGGGTCTCACGAAGCCGCCGTCA  468

Query  61   TTGTCTTTGGGAGGTGGGGTCGTCGCCGACGCCAGGCCGTCCAACACCAAGCCCAGAAAA  120
Sbjct  467  TTGTCTTTGGGAGGTGGGGTCGTCGCCGACGCCAGGCCGTCCAACACCAAGCCCAGAAAA  408

Query  121  GGGGGGCTTGAGAGGTGATAATGACGCTCGAACAGGCATGCCCTTCGGAATGCCAAAGGG  180
Sbjct  407  GGGGGGCTTGAGAGGTGATAATGACGCTCGAACAGGCATGCCCTTCGGAATGCCAAAGGG  348

Query  181  CGCAATGTGCGTTCAAAGATTCGATGACTCACTGGAATTCTGCAATTCGCATTACTTATC  240
Sbjct  347  CGCAATGTGCGTTCAAAGATTCGATGACTCACTGGAATTCTGCAATTCGCATTACTTATC  288

Query  241  GCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGC  300
Sbjct  287  GCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGC  228

Query  301  TTTTAATTAAAAAATAGTACTCAGAGTAGACACGTTTAATTAAGAATTTGTAAAGGTTGG  360
Sbjct  227  TTTTAATTAAAAAATAGTACTCAGAGTAGACACGTTTAATTAAGAATTTGTAAAGGTTGG  168

Query  361  CCACCGACGAGCGCGGGCCAGAGGACGGTTTGAAGGCCCCCCGGCGGTCTGccccccGG  420
Sbjct  167  CCACCGACGAGCGCGGGCCAGAGGACGGTTTGAAGGCCCCCCGGCGGTCTGCCCCCCCGG  108

Query  421  AGAGGGAGAGACGGGCTCGCCGAAGCAACGTGGGTCGGGTAAACAAAGGGTTGGGAGGTC  480
Sbjct  107  AGAGGGAGAGACGGGCTCGCCGAAGCAACGTGGGTCGGGTAAACAAAGGGTTGGGAGGTC  48

Query  481  GAGCCTATAAAAGACCCTAACTCGTTAATGATCCTTCCGCAGGTTCA  527
Sbjct  47   GAGCCTATAAAAGACCCTAACTCGTTAATGATCCTTCCGCAGGTTCA  1
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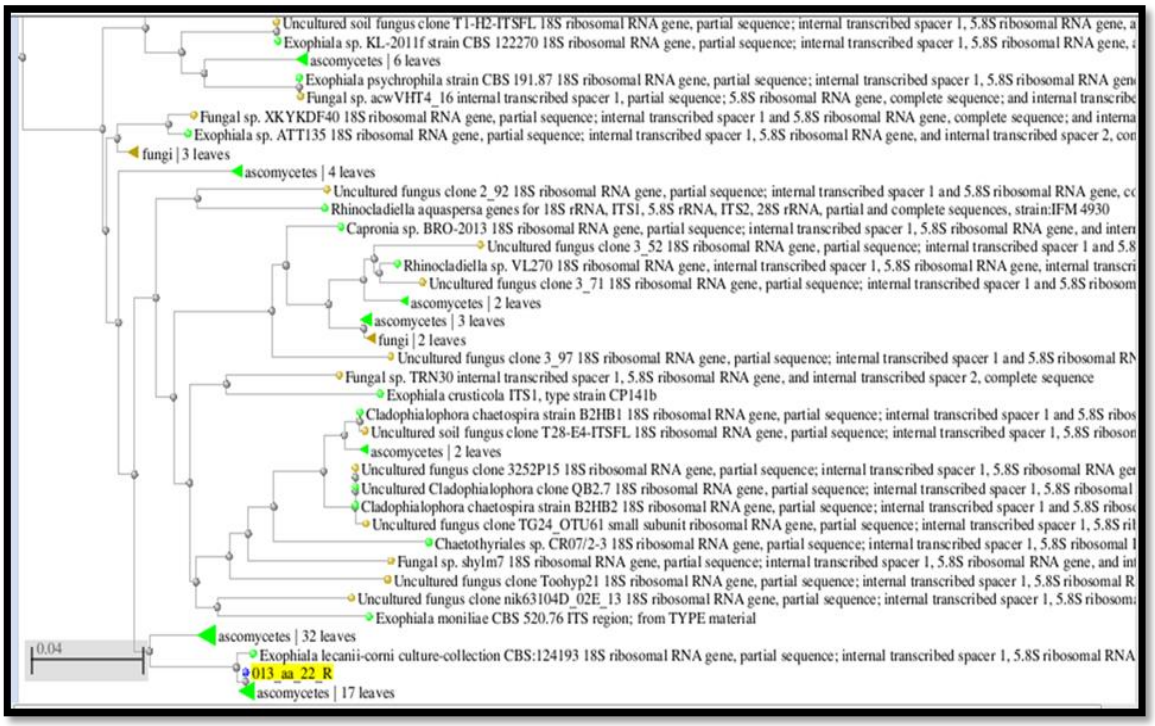
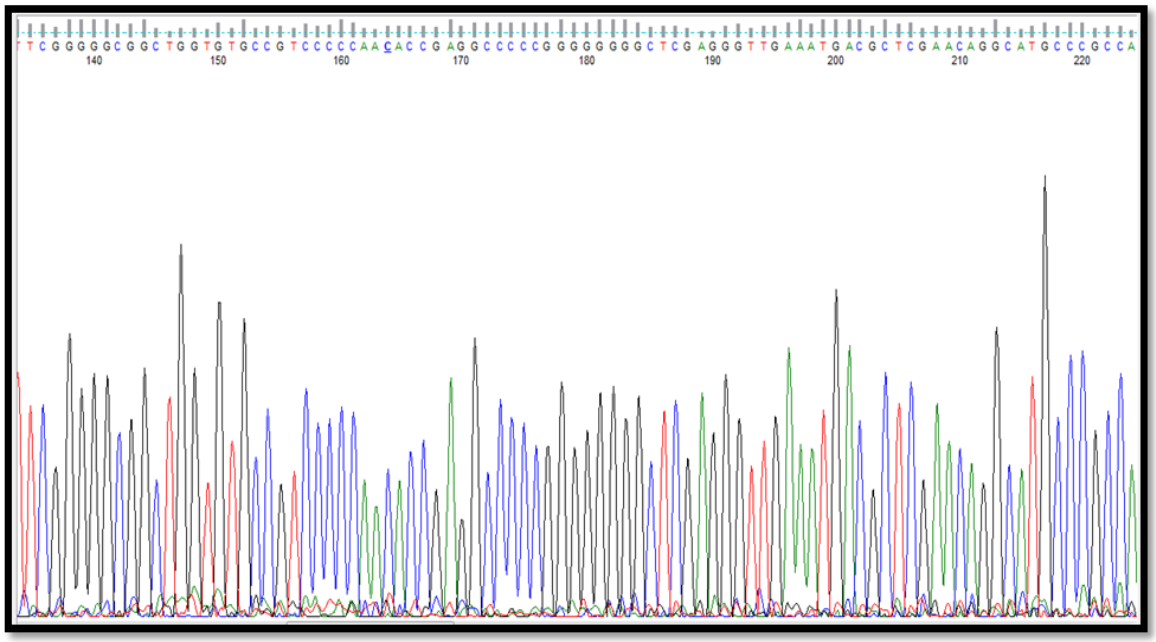


- ***Paecilomyces lilacinus***



Paecilomyces lilacinus genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, isolate Kw596W
Sequence ID: emb|FR751342.1|Length: 602Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
949 bits(1052)	0.0	526/526(100%)	0/526(0%)	Plus/Minus

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Query  1    GGGCGTTTTACGGCGTGACCGCCTCCGCGCTCCGGTGCGAGGTGTGTGCTACTACGCAGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526  GGGCGTTTTACGGCGTGACCGCCTCCGCGCTCCGGTGCGAGGTGTGTGCTACTACGCAGG  467

Query  61   GGAGGCTGCGGCGGGGTCGCCACTGCATTTCGGGGGCGGCTGGTGTGCCGTCCCCCAACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466  GGAGGCTGCGGCGGGGTCGCCACTGCATTTCGGGGGCGGCTGGTGTGCCGTCCCCCAACA  407

Query  121  CCGAGGCCCCCggggggggCTCGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406  CCGAGGCCCCCGGGGGGGGGCTCGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAG  347

Query  181  AATGCTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346  AATGCTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA  287

Query  241  CATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286  CATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTT  227

Query  301  GAAAGTTTTGATTCATTTGTTTTTGCTTGTGCAACTCAGAGAAGAAATTCCGCCCGCTGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226  GAAAGTTTTGATTCATTTGTTTTTGCTTGTGCAACTCAGAGAAGAAATTCCGCCCGCTGG  167

Query  361  GCGTAATGCAAGAGAGTTTggggtccctgcggcgggcgcctgggtccggcgccggcgcgg  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166  GCGTAATGCAAGAGAGTTTGGGGTCCCTGCGGCGGGCGCCTGGGTCCGGCGCCGGCGCGG  107

Query  421  gggcaggcggccgggggcgTTCCCGCCGAGGCAACTGAGGTAAGGTTCACAGTGGGTTTGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106  GGGCAGGCGGCCGGGGCGTTCCCGCCGAGGCAACTGAGGTAAGGTTCACAGTGGGTTTGG  47

Query  481  GAGTTGTATAACTCGGTAATGATCCCTCCGCAGGTTCACCTACGGA  526
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  46   GAGTTGTATAACTCGGTAATGATCCCTCCGCAGGTTCACCTACGGA  1
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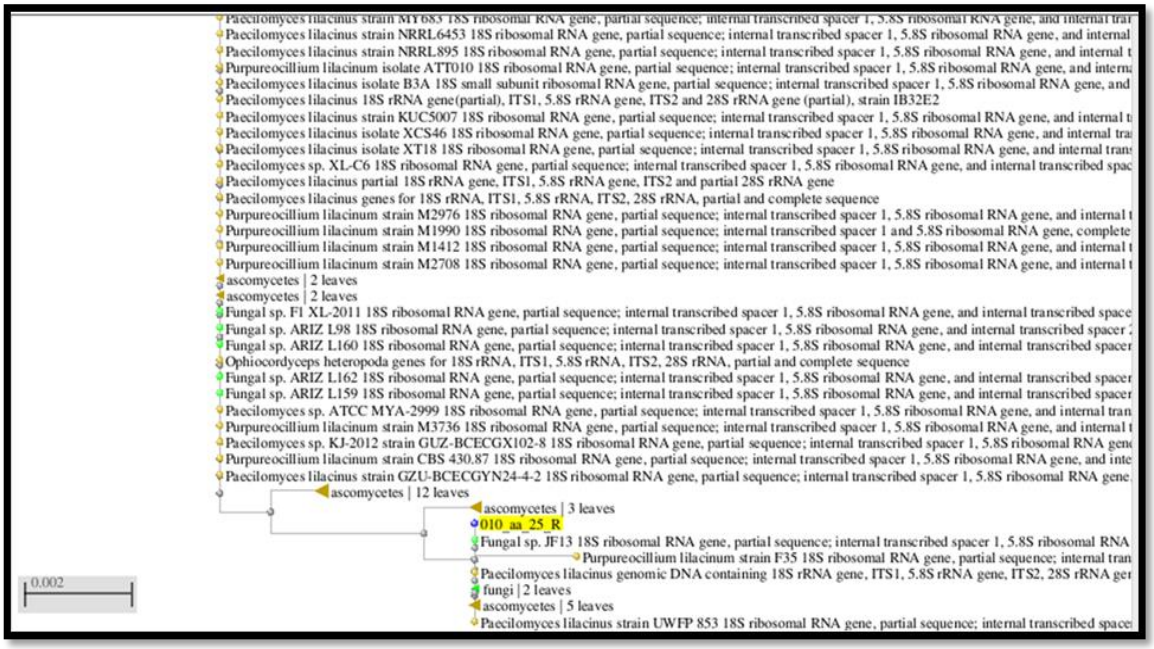
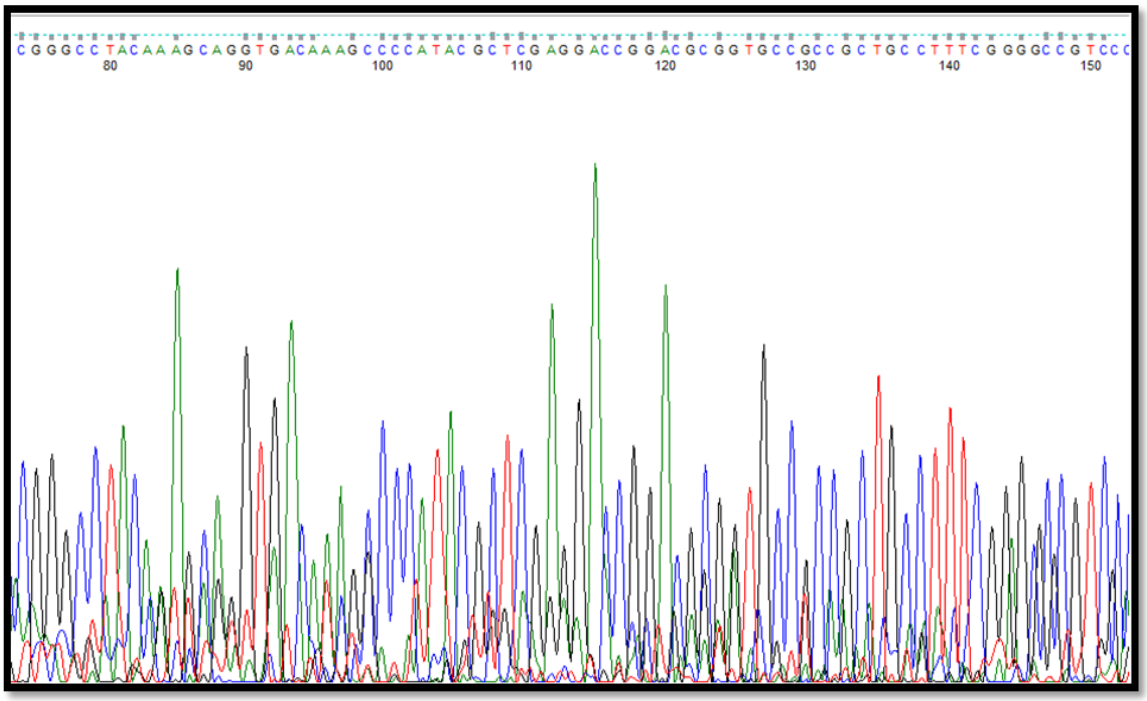


- ***Aspergillus fumigatus***



Aspergillus fumigatus gene for 18S ribosomal RNA, partial sequence, isolate: A.fumigatus Zag1

Sequence ID: dbj|AB976023.1|Length: 636Number of Matches: 2

Score	Expect	Identities	Gaps	Strand
881 bits(976)	0.0	504/515(98%)	0/515(0%)	Plus/Minus

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Query  1    AGTTGGGTGTCGGCTGGCGCCGGCCGGGCCTACAAAGCAGGTGACAAAGCCCCATACGCT  60
            ||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||||
Sbjct  594  AGTTGGGTGTCGGCTGGCGCCGGCCGGGCCTACAGAGCAGGTGACAAAGCCCCATACGCT  535

Query  61   CGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGGCCGTCCCCCGGGAGAGGGGGACG  120
            |||||||||||||||||||||||||||||||||| |||||||||||||||||||||||||
Sbjct  534  CGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGAGAGGGGGACG  475

Query  121  GGGGCCCAAGACACAATCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCC  180
            ||||||||| |||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  474  GGGGCCCAACACACAAGCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCC  415

Query  181  CGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414  CGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAAT  355

Query  241  TCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGANGCCGGAACCAATAGATCCGTT  300
            |||||||||||||||||||||||||||||||||||| |||||||||| |||||||||||
Sbjct  354  TCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTT  295

Query  301  GTTGAAAGTTTTAACTGATTACGATGATCAACTCAGACTGCATACTTTCAGAACAGCCTT  360
            |||||||||||||||||||||||||| |||||||||||||||||||||||||||||  ||
Sbjct  294  GTTGAAAGTTTTAACTGATTACGATAATCAACTCAGACTGCATACTTTCAGAACAGCGTT  235

Query  361  CATGTTGGGGTCTTcggcgggcgcgggcccgggggcgcaaggcctccccggcggccggcg  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||  ||
Sbjct  234  CATGTTGGGGTCTTCGGCGGGCGCGGGCCCGGGGGCGCAAGGCCTCCCCGGCGGCCGTCG  175

Query  421  aaacggcgggcccgccgAAGCAAAGAGGTACGATAGACACGGGTGGGAGGTTGGACCCAG  480
            ||||||||||||||||||||||||  ||||||||||||||||||||||||||||||||||
Sbjct  174  AAACGGCGGGCCCGCCGAAGCAACAAGGTACGATAGACACGGGTGGGAGGTTGGACCCAG  115

Query  481  AGGGCCCTCACTCGGTAATGATCCTTCCGCAGGTA  515
            |||||||||||||||||||||||||||||||||||
Sbjct  114  AGGGCCCTCACTCGGTAATGATCCTTCCGCAGGTA  80
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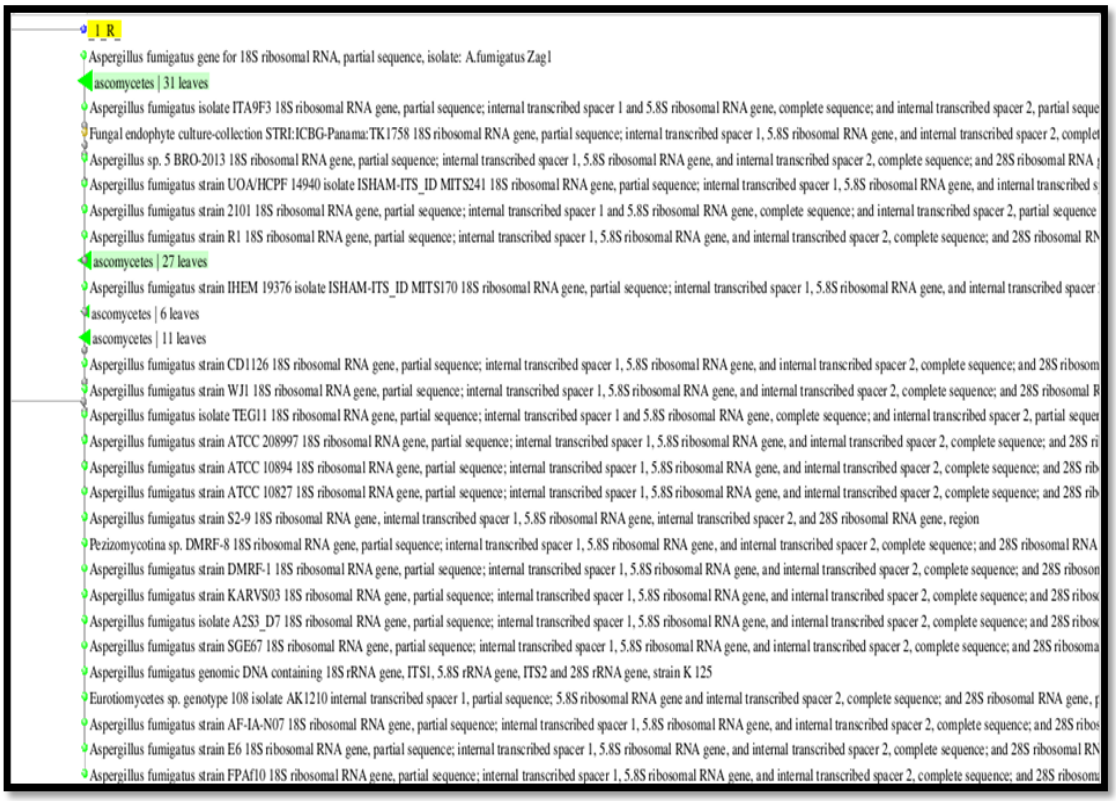
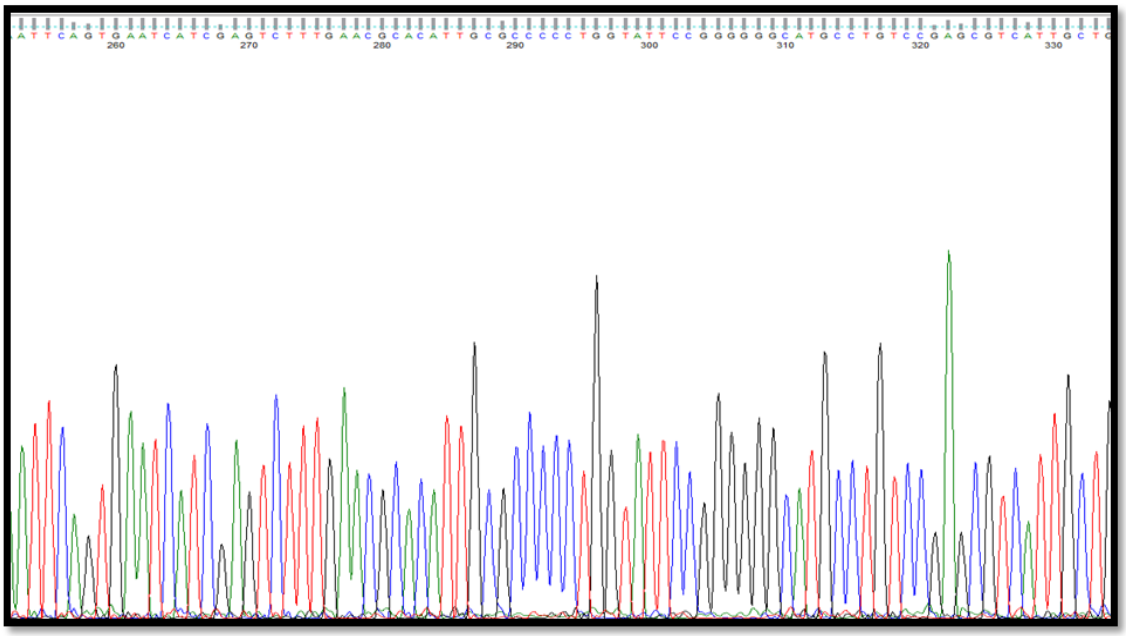


- ***Penicillium polonicum***



Penicillium polonicum isolate FZ-17 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

Sequence ID: gb|KF848936.1|Length: 555Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
924 bits(1024)	0.0	517/520(99%)	0/520(0%)	Plus/Plus

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Query  1    GTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTTACTGGCCGCCGGGGGGCTCACGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  36   GTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTTACTGGCCGCCGGGGGGCTCACGC  95

Query  61   CCCCGGGCCCGCGCCCGCCGAAGACACCCCCGAACTCTGTCTGAAGATTGAAGTCTGAGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  96   CCCCGGGCCCGCGCCCGCCGAAGACACCCCCGAACTCTGTCTGAAGATTGAAGTCTGAGT  155

Query  121  GAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  156  GAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG  215

Query  181  AACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  216  AACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTG  275

Query  241  AACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  276  AACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCC  335

Query  301  CTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCTCCGATTCCGGGGGACGGGCCCGAAAGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  336  CTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCTCCGATTCCGGGGGACGGGCCCGAAAGG  395

Query  361  CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  396  CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGC  455

Query  421  CCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCTCGGATCAGGTAGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  456  CCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCTCGGATCAGGTAGG  515

Query  481  GATACCCGCTGAACTTAAGCATATCATAAAGCAGGAGGAA  520
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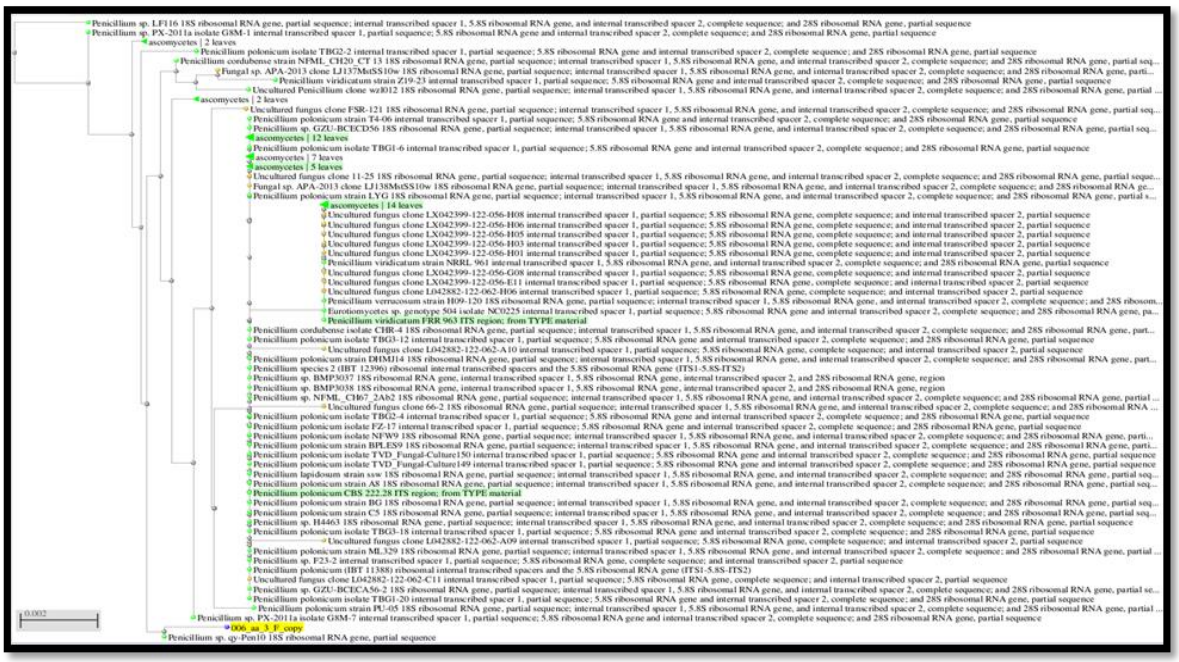
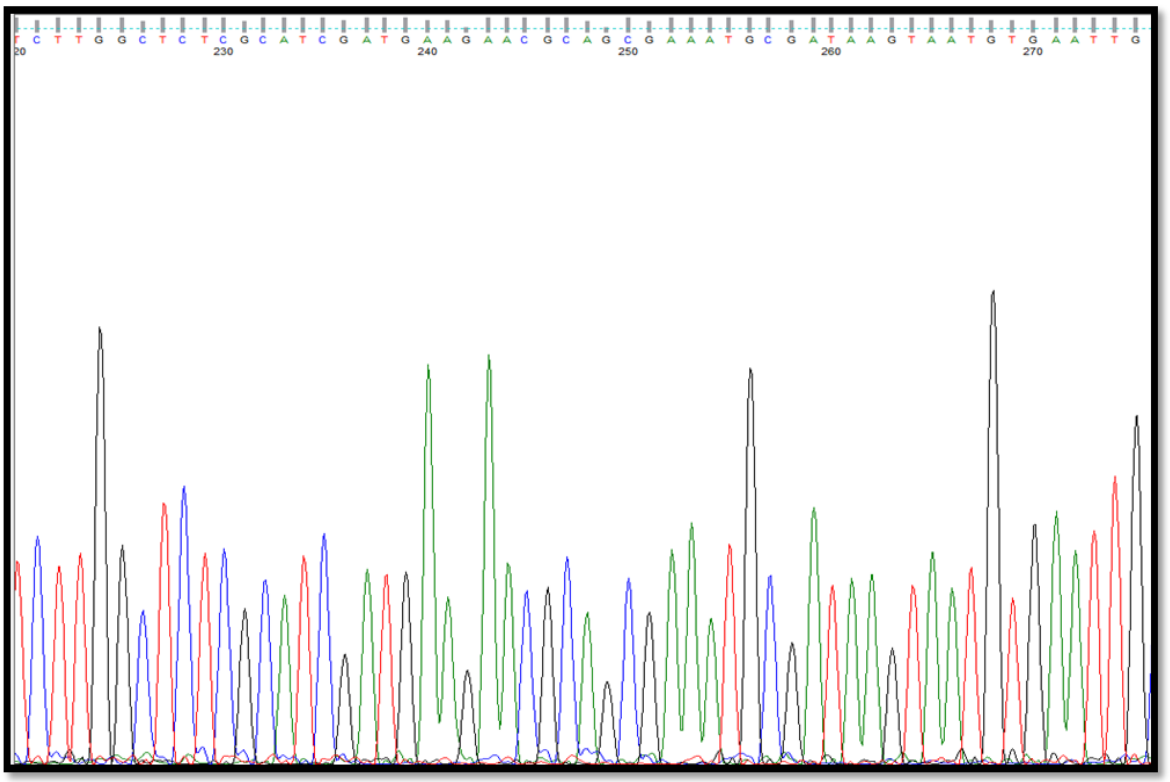


- ***Bjerkandera adusta***



Bjerkandera adusta strain B_a 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence

Sequence ID: gb|KF681360.1|Length: 620Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
993 bits(1100)	0.0	558/562(99%)	2/562(0%)	Plus/Plus

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Query  1    GCATGTGCACGCCTGTCTTCATCCACTCTCAACTTCTGTGCACTTTTCATAGGCCGGCTT  60
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Sbjct  58   GCATGTGCACGCCTGTCTTCATCCACTCTCAACTTCTGTGCACTTTTCATAGGCCGGCTT  117

Query  61   GTGGGTGCGCGTTCGCGTGCGTCTGTAGGTCTGGTTTATGCTTTATACTACAAACGAATC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  118  GTGGGTGCGCGTTCGCGTGCGTCTGTAGGTCTGGTTTATGCTTTA--CTACAAACGAATC  175

Query  121  AGTTTTAGAATGTCATACTTTGCTATAACGCAATTATATACAACTTTCAGCAACGGATCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  176  AGTTTTAGAATGTCATACTTTGCTATAACGCAATTATATACAACTTTCAGCAACGGATCT  235

Query  181  CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  236  CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA  295

Query  241  TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  296  TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG  355

Query  301  CCTGTTTGAGTCTCATGGAATTCTCAACCTTCGACTTTGTTGTCGGAGGCTTGGACTTGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  356  CCTGTTTGAGTCTCATGGAATTCTCAACCTTCGACTTTGTTGTCGGAGGCTTGGACTTGG  415

Query  361  AGGTCGTGTCGGCTCTCGTAGTCGACTCCTCTGAAATGCATTAGTGCGAACGTTACCAGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  416  AGGTCGTGTCGGCTCTCGTAGTCGACTCCTCTGAAATGCATTAGTGCGAACGTTACCAGC  475

Query  421  CGCTTCAGCGTGATAATTATCTGCGTTGCTGTGGAGGGTATGCTAGTGTTCGCGCTTCTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  476  CGCTTCAGCGTGATAATTATCTGCGTTGCTGTGGAGGGTATGCTAGTGTTCGCGCTTCTA  535

Query  481  ACCGTCTTCGGACAACATTTCTAAACTCTGAGCTCAAATCAGGTAGGACTACCCGCTGAA  540
            |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  536  ACCGTCTTCGGACAACATTTCTAGACTCTGAGCTCAAATCAGGTAGGACTACCCGCTGAA  595

Query  541  CTTAAGCATATCAATAAGCCGA  562
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Sbjct  596  CTTAAGCATATCAATAAGCGGA  617
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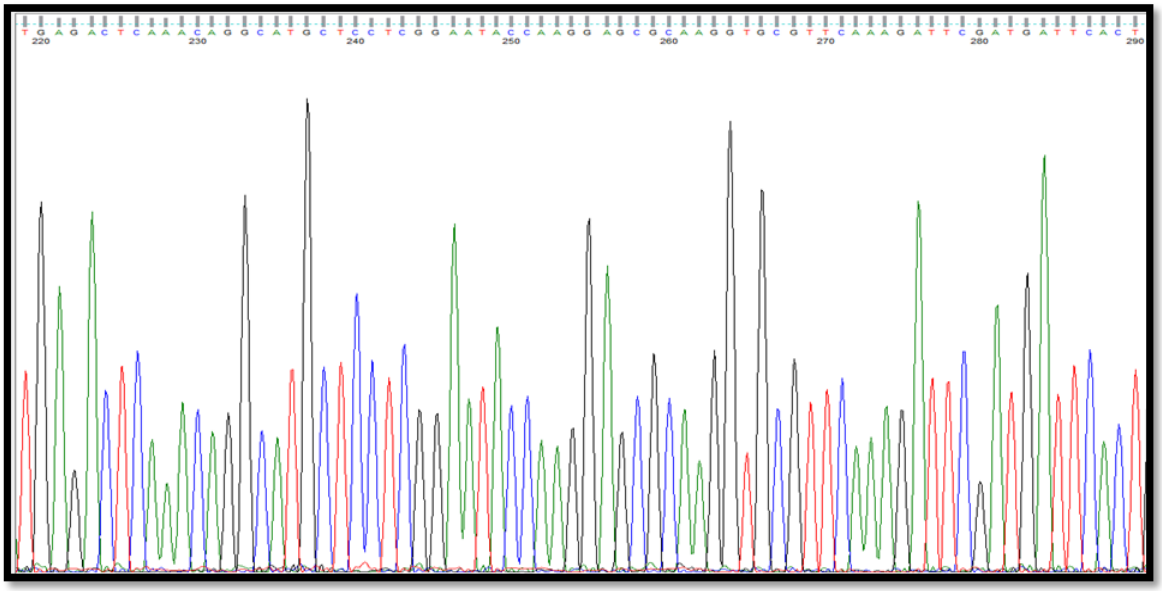
- ***Thanatephorus cucumeris***



Thanatephorus cucumeris strain IHB F 1913 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
Sequence ID: gb|KF381087.1|Length: 632Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
994 bits(1102)	0.0	551/551(100%)	0/551(0%)	Plus/Minus

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Query  1    GAAATTTGTCCGAAGACGGTTAGAAGCGTGAACACTAGAATACCCTCCACAGCAACGCAG  60
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Sbjct  567  GAAATTTGTCCGAAGACGGTTAGAAGCGTGAACACTAGAATACCCTCCACAGCAACGCAG  508

Query  61   ATAATTATCACGCTGAAGCGGCTGGTAACGTTCGCACTAATGCATTTCAGAGGAGCCGAC  120
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Sbjct  507  ATAATTATCACGCTGAAGCGGCTGGTAACGTTCGCACTAATGCATTTCAGAGGAGCCGAC  448

Query  121  TACGAGAGCCGGCACGACCTCCAAGTCCAAGCCTTCATCAATAAAGCTGAAGGTTGAGAA  180
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Sbjct  447  TACGAGAGCCGGCACGACCTCCAAGTCCAAGCCTTCATCAATAAAGCTGAAGGTTGAGAA  388

Query  181  TTCCATGAGACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387  TTCCATGAGACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAA  328

Query  241  AGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  327  AGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCT  268

Query  301  TCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAAATTGCGTTATAGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  267  TCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAAATTGCGTTATAGC  208

Query  361  AAAGTATGACATTCTAAAACTGAATCGTTTGTAATAAAGCATAAGCCCGACACCTACAAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  207  AAAGTATGACATTCTAAAACTGAATCGTTTGTAATAAAGCATAAGCCCGACACCTACAAG  148

Query  421  TGCGCGAACGCACCCACAAGCCGGCCTATGAAAAGTGCACAGAAGTTGAGAGTGGATGAG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  147  TGCGCGAACGCACCCACAAGCCGGCCTATGAAAAGTGCACAGAAGTTGAGAGTGGATGAG  88

Query  481  ACAGGCGTGCACATGCCCTTGCGAGCCAGCAGACAACCCGTTCAAAACTCGATAATGATC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  87   ACAGGCGTGCACATGCCCTTGCGAGCCAGCAGACAACCCGTTCAAAACTCGATAATGATC  28

Query  541  CTTCCGCAGGT  551
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Sbjct  27   CTTCCGCAGGT  17
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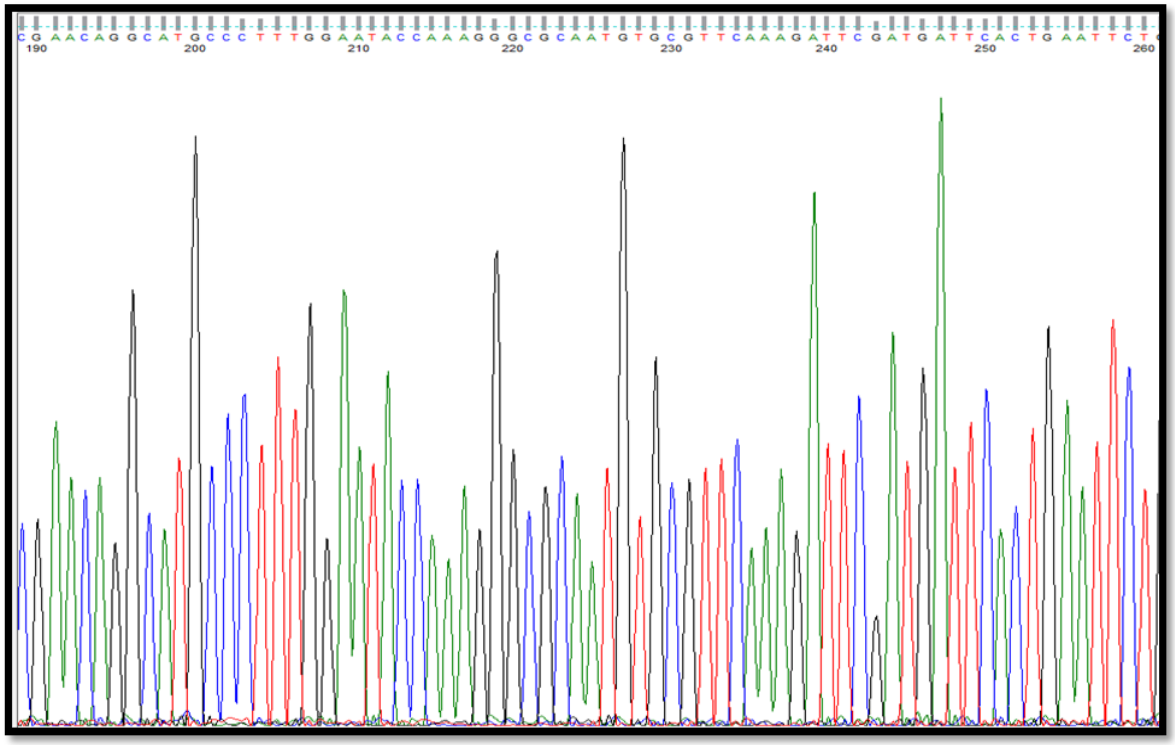
- ***Alternaria obovoidea***



Alternaria obovoidea 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

Sequence ID: gb|KC466541.1|Length: 598Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
921 bits(1020)	0.0	510/510(100%)	0/510(0%)	Plus/Minus

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Query  1    GTGGATGCTGACCTTGGCTGGAAGAGAGCGCGACTTGTGCTGCGCTCCGAAACCAGTAGG  60
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Sbjct  522  GTGGATGCTGACCTTGGCTGGAAGAGAGCGCGACTTGTGCTGCGCTCCGAAACCAGTAGG  463

Query  61   CCGGCTGCCAATAACTTTAAGGCGAGTCCCCAGCGAACTGGAGACAAGACGCCCAACACC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  462  CCGGCTGCCAATAACTTTAAGGCGAGTCCCCAGCGAACTGGAGACAAGACGCCCAACACC  403

Query  121  AAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  402  AAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGG  343

Query  181  GCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  342  GCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATC  283

Query  241  GCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  282  GCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAA  223

Query  301  TTATTATTATTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGGTTTGTCCTTGTG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  222  TTATTATTATTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGGTTTGTCCTTGTG  163

Query  361  GTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACACGGGTGAATAATTCAGCA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  162  GTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACACGGGTGAATAATTCAGCA  103

Query  421  AGGCTGGCCCCAACAGCGCACGCCGCAAAGCAACGCACTGCTGGGGGGAGTCCAGCCCGC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  102  AGGCTGGCCCCAACAGCGCACGCCGCAAAGCAACGCACTGCTGGGGGGAGTCCAGCCCGC  43

Query  481  TTTCATATTGTGTAATGATCCCTCCGCAGG  510
            |||||||||||||||||||||||||||||
Sbjct  42   TTTCATATTGTGTAATGATCCCTCCGCAGG  13
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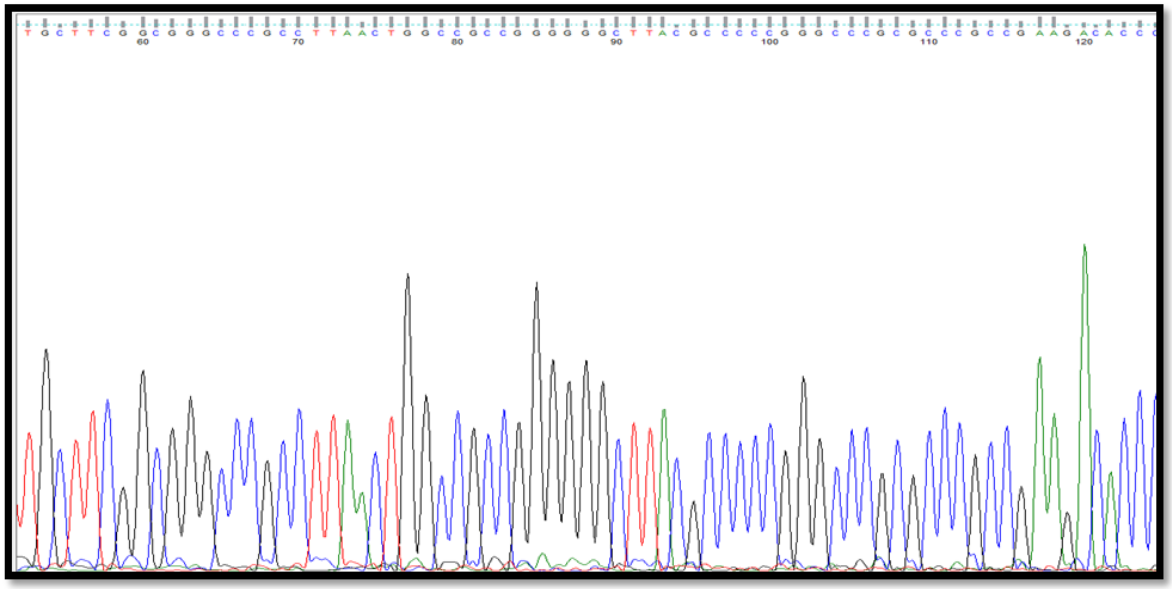
- ***Penicillium commune***



Penicillium commune strain DHMJ04 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

Sequence ID: gb|JN986756.1|Length: 540Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
931 bits(1032)	0.0	518/519(99%)	0/519(0%)	Plus/Plus

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Query  1    GTTTATTTTACCTTGTTGCTTcggcgggcccgccttaactggccgccgggggggcttacgc  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  22   GTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCCGGGGGGCTTACGC  81

Query  61   ccccgggcccgcgcccgccgAAGACACCCTCGAACTCTGTCTGAAGATTGTAGTCTGAGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  82   CCCCGGGCCCGCGCCCGCCGAAGACACCCTCGAACTCTGTCTGAAGATTGTAGTCTGAGT  141

Query  121  GAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  142  GAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG  201

Query  181  AACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  202  AACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTG  261

Query  241  AACGCACATTGCGCCCCCTGGTATTCCGGGGGGGCATGCCTGTCCGAGCGTCATTTCTGCC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  262  AACGCACATTGCGCCCCCTGGTATTCCGGGGGGGCATGCCTGTCCGAGCGTCATTTCTGCC  321

Query  301  CTCAAGCACGGCTTGTGTGTTGGGCCCCGTCCTCCGATCCCGGGGGACGGGCCCGAAAGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  322  CTCAAGCACGGCTTGTGTGTTGGGCCCCGTCCTCCGATCCCGGGGGACGGGCCCGAAAGG  381

Query  361  CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  382  CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGC  441

Query  421  CCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCTCGGATCAGGTAGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  442  CCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCTCGGATCAGGTAGG  501

Query  481  GATACCCGCTGAACTTAAGCATATCAATAACAGGAGGAA  519
            |||||||||||||||||||||||||| ||||||||
Sbjct  502  GATACCCGCTGAACTTAAGCATATCAATAAGAGGAGGAA  540
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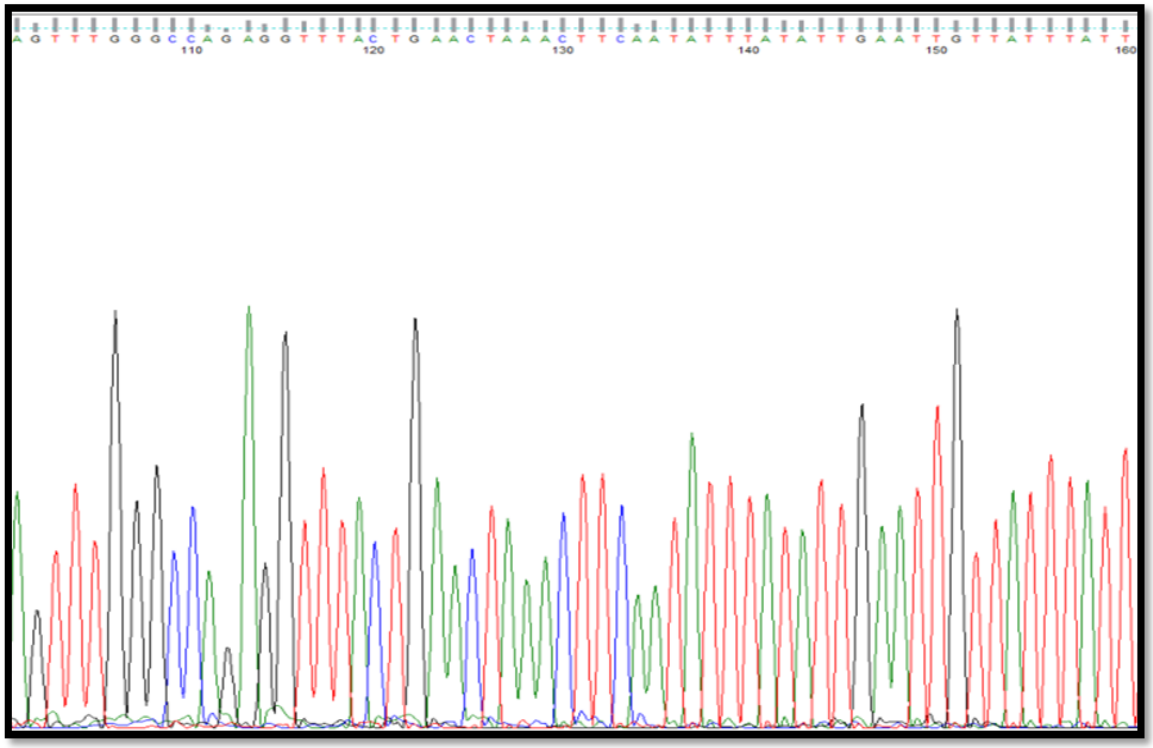
- ***Debaryomyces hansenii***



Debaryomyces hansenii 26S ribosomal RNA gene, partial sequence
Sequence ID: gb|KC111880.1|Length: 614Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
1025 bits(1136)	0.0	572/575(99%)	0/575(0%)	Plus/Plus

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Query  1    GAAAAACCTTACACACAGTGTTTTTTGTTATTACAAGAACTTTTGCTTTGGTCTGGACTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  40   GAAAAACCTTACACACAGTGTTTTTTGTTATTACAAGAACTTTTGCTTTGGTCTGGACTA  99

Query  61   GAAATAGTTTGGGCCAGAGGTTTACTGAACTAAACTTCAATATTTATATTGAATTGTTAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  100  GAAATAGTTTGGGCCAGAGGTTTACTGAACTAAACTTCAATATTTATATTGAATTGTTAT  159

Query  121  TTATTTAATTGTCAATTTGTTGATTAAATTCAAAAAATCTTCAAAACTTTCAACAACGGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  160  TTATTTAATTGTCAATTTGTTGATTAAATTCAAAAAATCTTCAAAACTTTCAACAACGGA  219

Query  181  TCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  220  TCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCA  279

Query  241  GATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  280  GATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGC  339

Query  301  ATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCTTCGGGTTTGGTATTGAGTGATACTCT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  340  ATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCTTCGGGTTTGGTATTGAGTGATACTCT  399

Query  361  TAGTCGAACTAGGCGTTTGCTTGAAATGTATTGGCATGAGTGGTACTGGATAGTGCTATA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  400  TAGTCGAACTAGGCGTTTGCTTGAAATGTATTGGCATGAGTGGTACTGGATAGTGCTATA  459

Query  421  TGACTTTCAATGTATTAGGTTTATCCAACTCGTTGAATAGTTTAATGGTATATTTCTCGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  460  TGACTTTCAATGTATTAGGTTTATCCAACTCGTTGAATAGTTTAATGGTATATTTCTCGG  519

Query  481  TATTCTAGGCTCGGCCTTACAATATAACAAACAAGTTTGACCTCAAATCAGGTAGGANTA  540
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  520  TATTCTAGGCTCGGCCTTACAATATAACAAACAAGTTTGACCTCAAATCAGGTAGGATTA  579

Query  541  CCCGCTGAACTTAAGCATATCAATAACCGGAAGAA  575
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Sbjct  580  CCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  614
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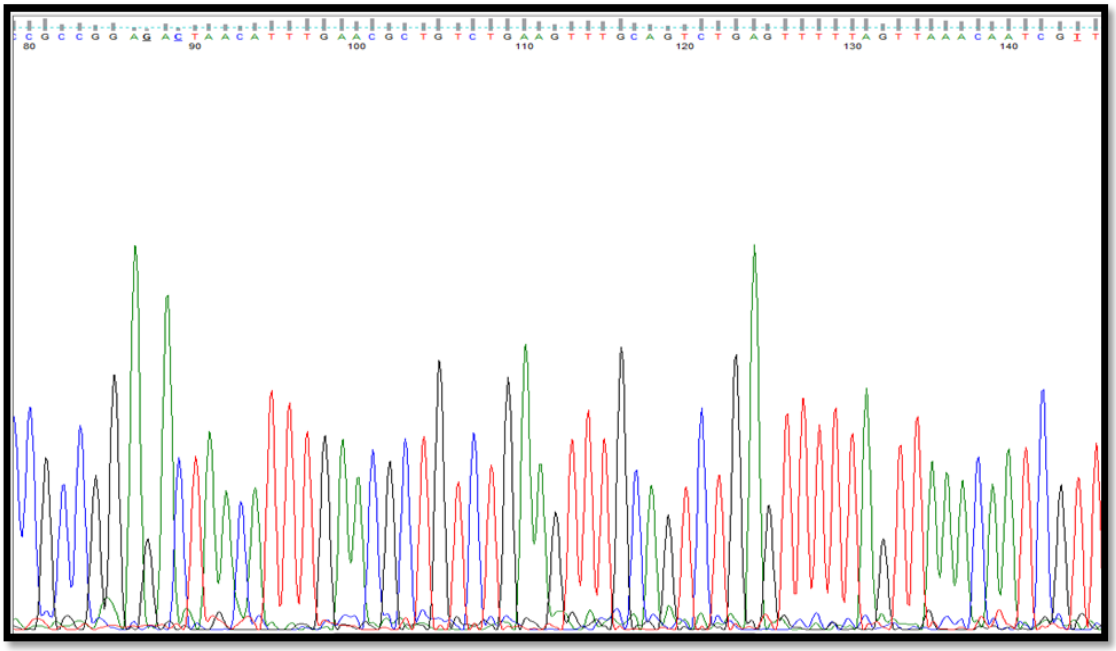
- ***Aspergillus cristatus***



Aspergillus cristatus strain CGMCC3.6088 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
Sequence ID: gb|KM388854.1|Length: 1082Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
884 bits(980)	0.0	490/490(100%)	0/490(0%)	Plus/Plus

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Query  1    GTGTCTATCTGTACCCTGTTGCTTCGGCGTGGCCACGGCCCGCCGGAGACTAACATTTGA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  47   GTGTCTATCTGTACCCTGTTGCTTCGGCGTGGCCACGGCCCGCCGGAGACTAACATTTGA  106

Query  61   ACGCTGTCTGAAGTTTGCAGTCTGAGTTTTTAGTTAAACAATCGTTAAAACTTTCAACAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  107  ACGCTGTCTGAAGTTTGCAGTCTGAGTTTTTAGTTAAACAATCGTTAAAACTTTCAACAA  166

Query  121  CGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAATTAATGTGAAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  167  CGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAATTAATGTGAAT  226

Query  181  TGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  227  TGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGG  286

Query  241  GGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCTTCCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  287  GGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCTTCCG  346

Query  301  TCCCTGGCAACGGGGACGGGCCCAAAAGGCAGTGGCGGCACCATGTCTGGTCCTCGAGCG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  347  TCCCTGGCAACGGGGACGGGCCCAAAAGGCAGTGGCGGCACCATGTCTGGTCCTCGAGCG  406

Query  361  TATGGGGCTTTGTCACCCGCTCCCGTAGGTCCAGCTGGCAGCTAGCCTCGCAACCAATCT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  407  TATGGGGCTTTGTCACCCGCTCCCGTAGGTCCAGCTGGCAGCTAGCCTCGCAACCAATCT  466

Query  421  TTTTAACCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  467  TTTTAACCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA  526

Query  481  AGCGGAGGAA  490
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Sbjct  527  AGCGGAGGAA  536
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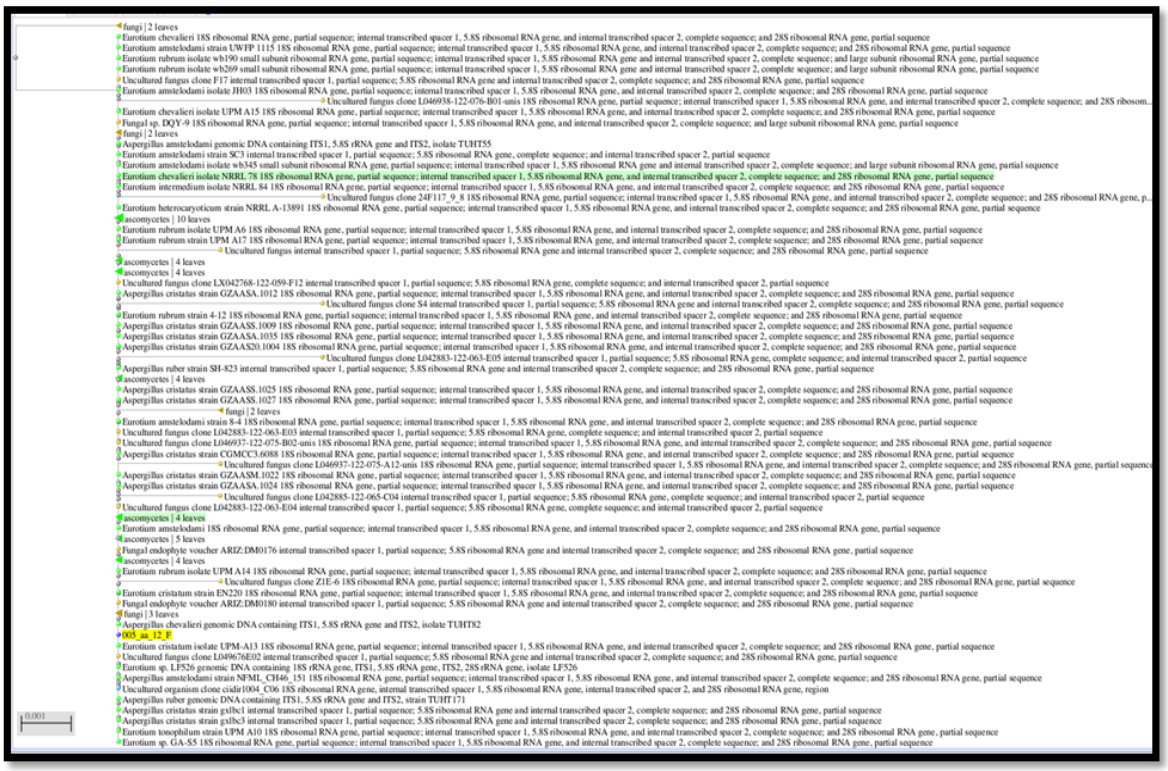
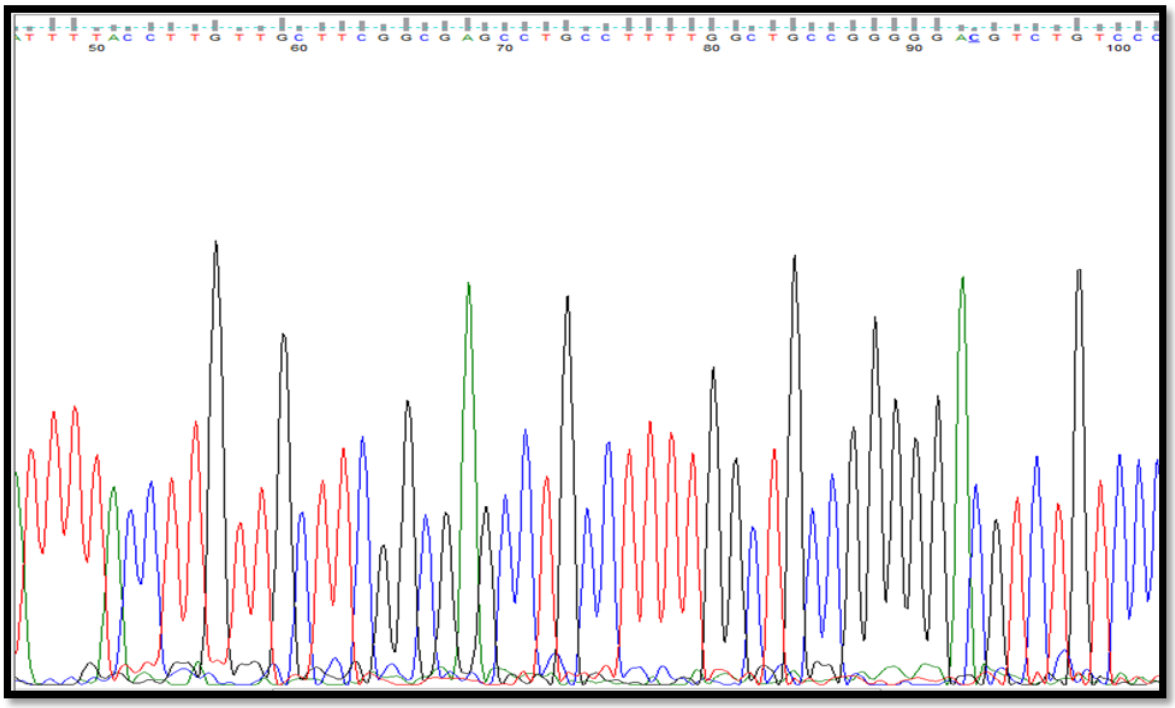


- ***Penicillium brevicompactum***



Penicillium brevicompactum strain FRR 66 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

Sequence ID: gb|AY373897.1|Length: 605Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
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Query  1    GTGTTTATTTTACCTTGTTGCTTCGGCGAGCCTGCCTTTTGGCTGCCGGGGGACGTCTGT  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  66   GTGTTTATTTTACCTTGTTGCTTCGGCGAGCCTGCCTTTTGGCTGCCGGGGGACGTCTGT  125

Query  61   CCCCGGGTCCGCGCTCGCCGAAGACACCTTAGAACTCTGTCTGAAGATTGTAGTCTGAGA  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  126  CCCCGGGTCCGCGCTCGCCGAAGACACCTTAGAACTCTGTCTGAAGATTGTAGTCTGAGA  185

Query  121  TTAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  TTAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG  245

Query  181  AACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTT  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  AACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTT  305

Query  241  GAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGC  300
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Sbjct  306  GAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGC  365

Query  301  CCTCAAGCACGGCTTGTGTGTTGGGCTCCGTCCTCCTTCCGGGGGGACGGGCCCGAAAGGC  360
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  366  CCTCAAGCACGGCTTGTGTGTTGGGCTCCGTCCTCCTTCCGGGGGACGGGCCCGAAAGGC  425

Query  361  AGCGGCGGCACCGCGTCCGGTCCTCAAGCGTATGGGGCTTTGTCACCCGCTTTGTAGGAC  420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  426  AGCGGCGGCACCGCGTCCGGTCCTCAAGCGTATGGGGCTTTGTCACCCGCTTTGTAGGAC  485

Query  421  TGGCCGGCGCCTGCCGATCAACCAAACTTTTTTCCAGGTTGACCTCGGATCAGGTAGGGA  480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  486  TGGCCGGCGCCTGCCGATCAACCAAACTTTTTTCCAGGTTGACCTCGGATCAGGTAGGGA  545

Query  481  TACCCGCTGAACTTAAGCATATCAATAAGCGGAAGA  516
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Sbjct  546  TACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA  581
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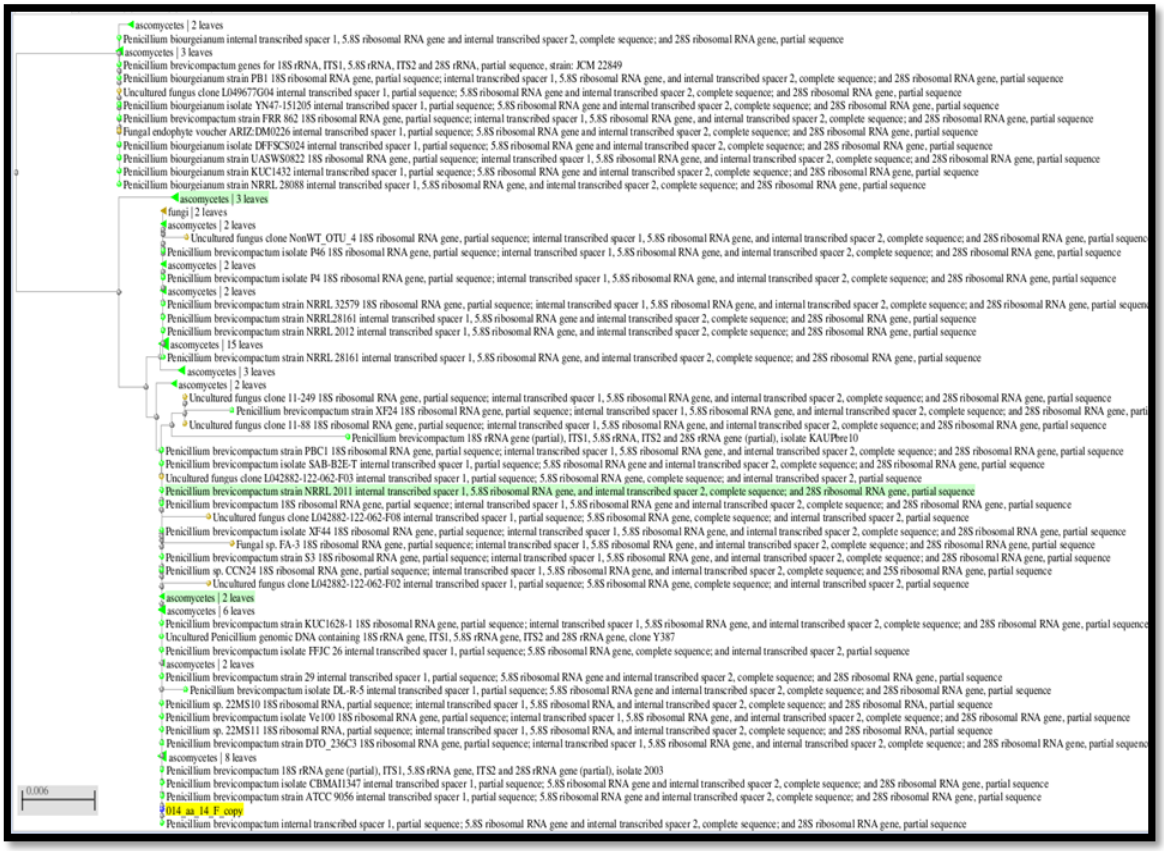
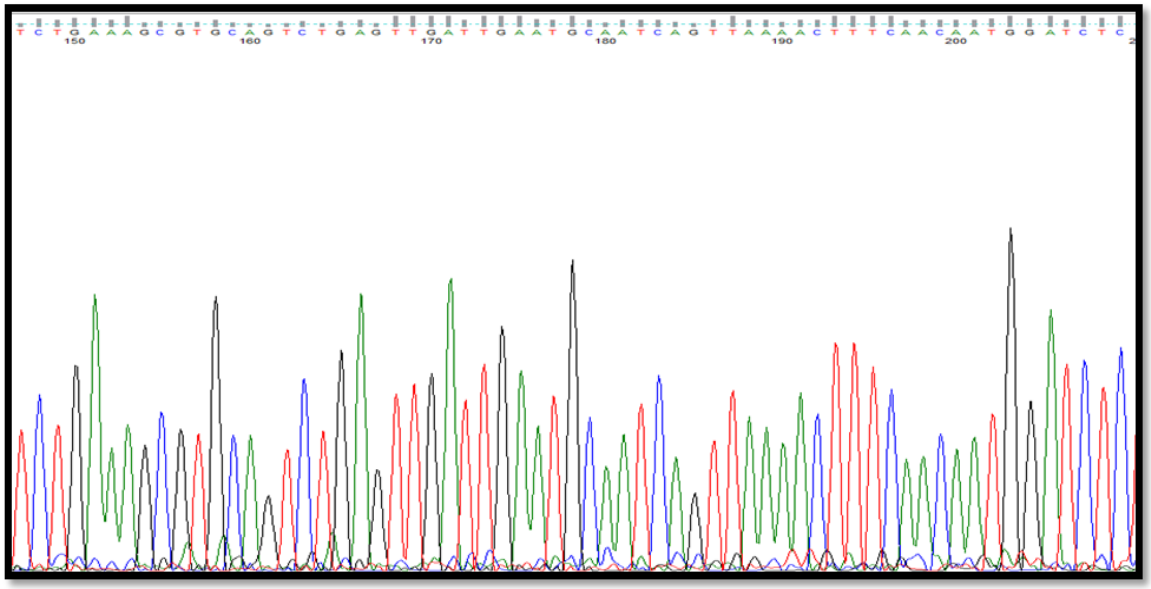


- ***Aspergillus niger***



Aspergillus niger strain YMA120 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

Sequence ID: gb|JF436884.1|Length: 560Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
957 bits(1060)	0.0	531/532(99%)	0/532(0%)	Plus/Plus

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Query  1    GTCTATTATACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCgggggggCGCCT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  29   GTCTATTATACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCT  88

Query  61   TTGCCCCCCGGGCCCGTGCCCGCCGGANACCCCAACACGAACACTGTCTGAAAGCGTGCA  120
            |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  89   TTGCCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCA  148

Query  121  GTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  GTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGC  208

Query  181  ATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  ATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCA  268

Query  241  TCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  TCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCG  328

Query  301  TCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  TCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGAC  388

Query  361  GGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACA  448

Query  421  TGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTTTTTCCAGGTTGACC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  449  TGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTTTTTCCAGGTTGACC  508

Query  481  TCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGGAGG  532
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
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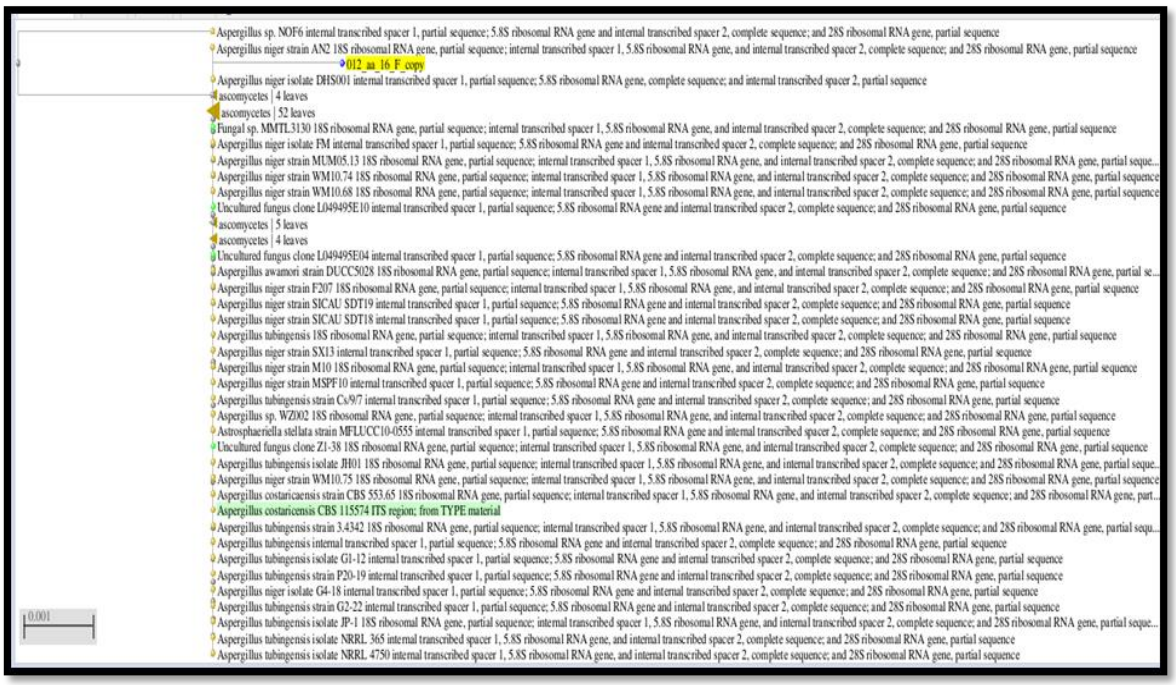
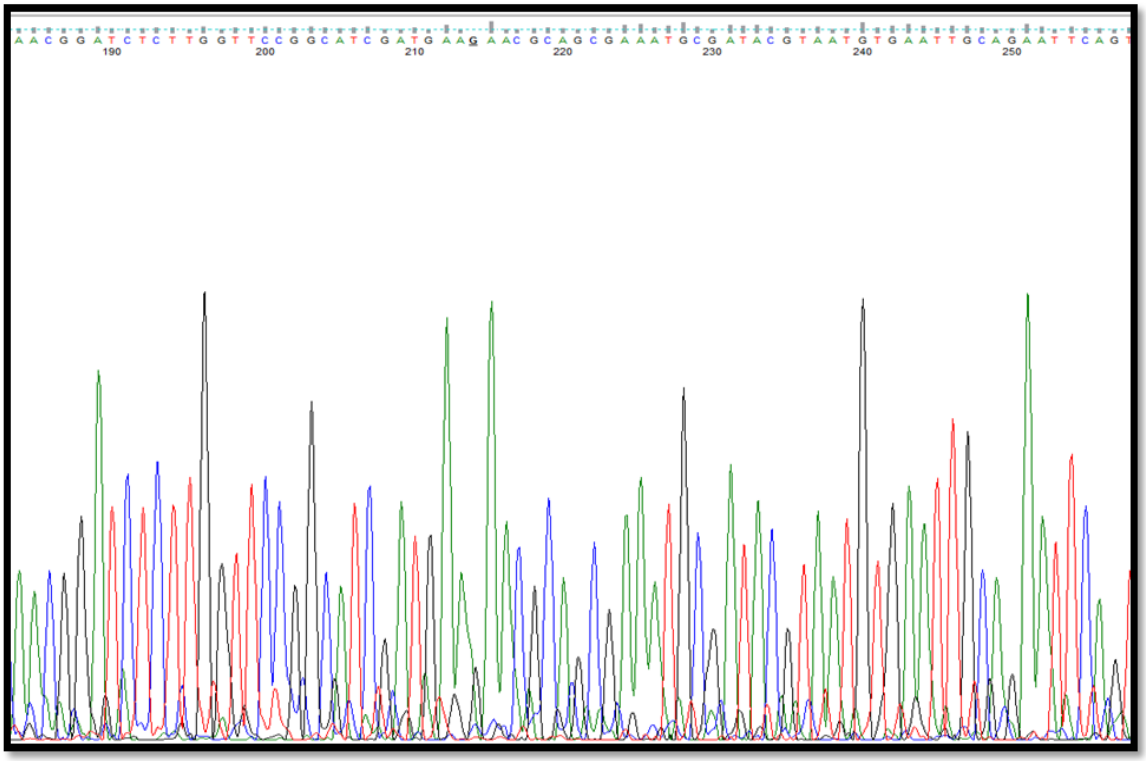


- ***Penicillium olsonii***



Penicillium olsonii strain 1.17 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

Sequence ID: gb|KM265447.1|Length: 592Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
933 bits(1034)	0.0	517/517(100%)	0/517(0%)	Plus/Plus

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Query  1    GTGTTTATTTTACCTTGTTGCTTCGGCGAGCCTGCCTTCGGGCTGCCGGGGGGCATCTGC  60
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Sbjct  75   GTGTTTATTTTACCTTGTTGCTTCGGCGAGCCTGCCTTCGGGCTGCCGGGGGGCATCTGC  134

Query  61   CCCCGGGTCCGCGCTCGCCGGAGACACCTTGAACTCTGTCTGAAGATTGTAGTCTGAGAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  135  CCCCGGGTCCGCGCTCGCCGGAGACACCTTGAACTCTGTCTGAAGATTGTAGTCTGAGAC  194

Query  121  AAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  195  AAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGA  254

Query  181  ACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  255  ACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTG  314

Query  241  AACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  315  AACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCC  374

Query  301  CTCAAGCACGGCTTGTGTGTTGGGCTCCGTCCTCCTCTTCTGGGGGGACGGGCCCCGAAAGGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  375  CTCAAGCACGGCTTGTGTGTTGGGCTCCGTCCTCCTCTTCTGGGGGGACGGGCCCCGAAAGGC  434

Query  361  AGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435  AGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGAC  494

Query  421  TGGCCGGCGCCTGCCGATCAACCAAACTTTTTTCCAGGTTGACCTCGGATCAGGTAGGGA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  495  TGGCCGGCGCCTGCCGATCAACCAAACTTTTTTCCAGGTTGACCTCGGATCAGGTAGGGA  554

Query  481  TACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  517
            |||||||||||||||||||||||||||||||||||||
Sbjct  555  TACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  591
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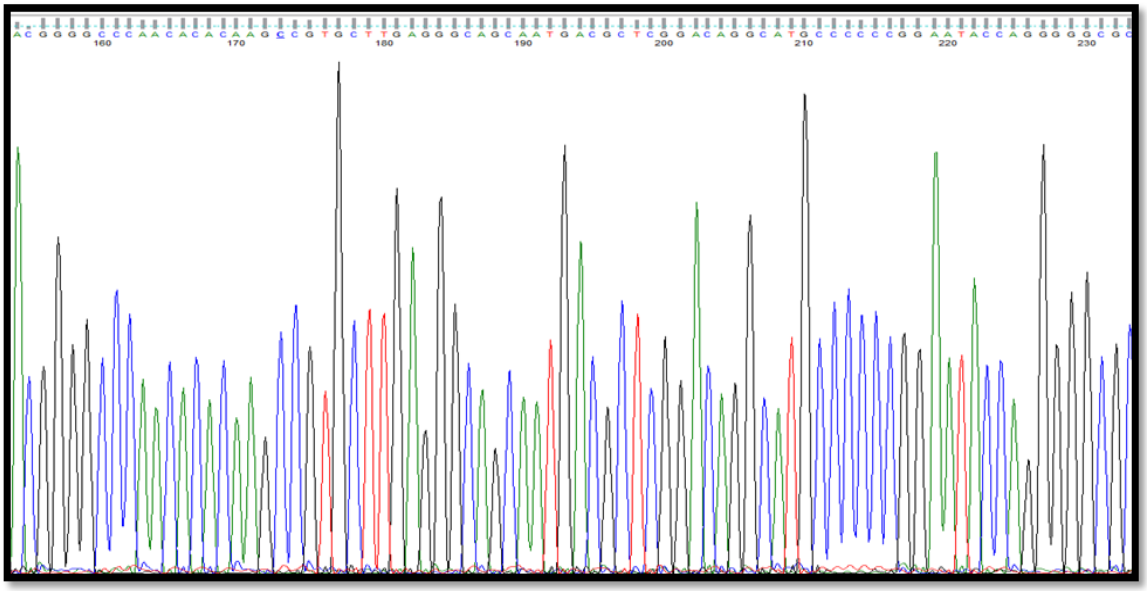
- ***Penicillium chrysogenum***



Penicillium chrysogenum isolate FFJaC 12 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
Sequence ID: gb|KM396379.1|Length: 574Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
904 bits(1002)	0.0	501/501(100%)	0/501(0%)	Plus/Minus

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Query  1    GGGTTGATCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATACGCTCG  60
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Sbjct  509  GGGTTGATCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATACGCTCG  450

Query  61   AGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGATCGGAGGACGGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  449  AGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGATCGGAGGACGGG  390

Query  121  GCCCAACACACAAGCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  389  GCCCAACACACAAGCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGG  330

Query  181  AATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTTGCAATTCAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  329  AATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTTGCAATTCAC  270

Query  241  ATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  269  ATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTG  210

Query  301  AAAGTTTTAAATAATTTATATTTTCACTCAGACTACAATCTTCAGACAGAGTTCGAGGGT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  209  AAAGTTTTAAATAATTTATATTTTCACTCAGACTACAATCTTCAGACAGAGTTCGAGGGT  150

Query  361  GTCTTcggcgggcgcgggcccgggggcgtaagccccccggcggccagttaaggcgggccc  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  149  GTCTTCGGCGGGCGCGGGCCCGGGGGCGTAAGCCCCCCGGCGGCCAGTTAAGGCGGGCCC  90

Query  421  gccgAAGCAACAAGGTAAAATAAACACGGGTGGGAGGTTGGACCCAGAGGGCCCTCACTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  89   GCCGAAGCAACAAGGTAAAATAAACACGGGTGGGAGGTTGGACCCAGAGGGCCCTCACTC  30

Query  481  GGTAATGATCCTTCCGCAGGT  501
            |||||||||||||||||||||
Sbjct  29   GGTAATGATCCTTCCGCAGGT  9
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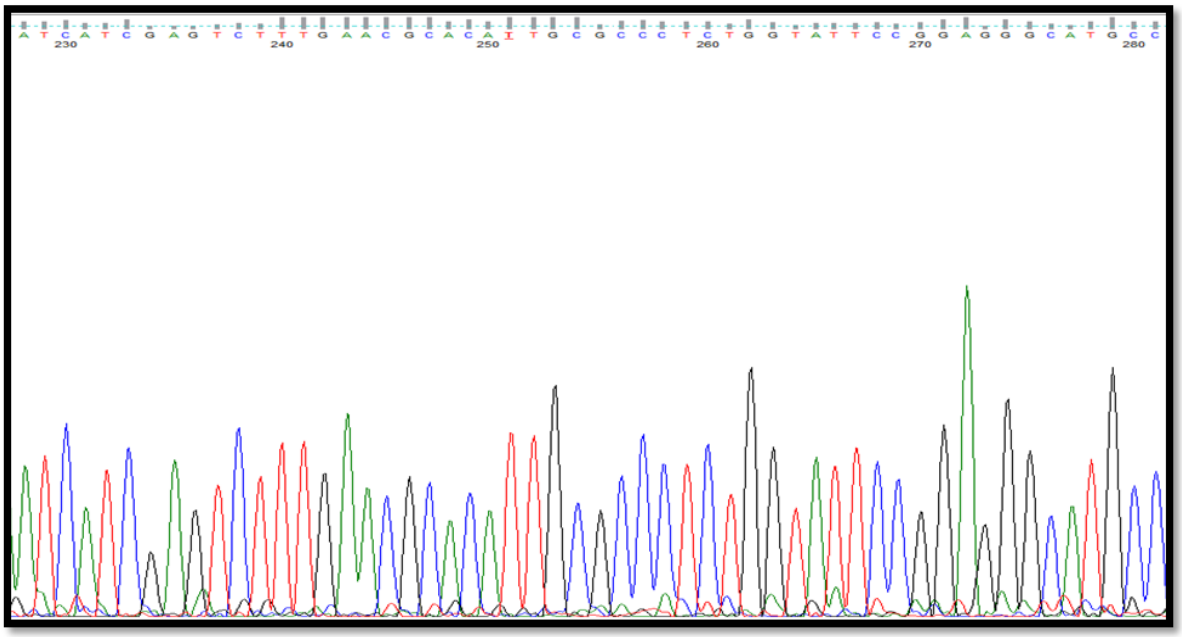
- ***Penicillium citrinum***



Penicillium citrinum 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

Sequence ID: gb|KM491892.1|Length: 861Number of Matches: 2

Score	Expect	Identities	Gaps	Strand
872 bits(966)	0.0	483/483(100%)	0/483(0%)	Plus/Plus

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Query  1    GCCCGAACCTATGTTGCCTCGGCGGGGCCCCGCGCCCGCCGACGGCCCCCCTGAACGCTGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  190  GCCCGAACCTATGTTGCCTCGGCGGGGCCCCGCGCCCGCCGACGGCCCCCCTGAACGCTGT  249

Query  61   CTGAAGTTGCAGTCTGAGACCTATAACGAAATTAGTTAAAACTTTCAACAACGGATCTCT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  CTGAAGTTGCAGTCTGAGACCTATAACGAAATTAGTTAAAACTTTCAACAACGGATCTCT  309

Query  121  TGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATT  369

Query  181  CAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  CAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCC  429

Query  241  TGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTcccccccGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCCCCCGC  489

Query  301  CGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  CGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGC  549

Query  361  TTCGTCACCCGCTCTAGTAGGCCCGGCCGGCGCCAGCCGACCCCCAACCTTTAATTATCT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  TTCGTCACCCGCTCTAGTAGGCCCGGCCGGCGCCAGCCGACCCCCAACCTTTAATTATCT  609

Query  421  CAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  610  CAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAG  669

Query  481  GAA  483
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Sbjct  670  GAA  672
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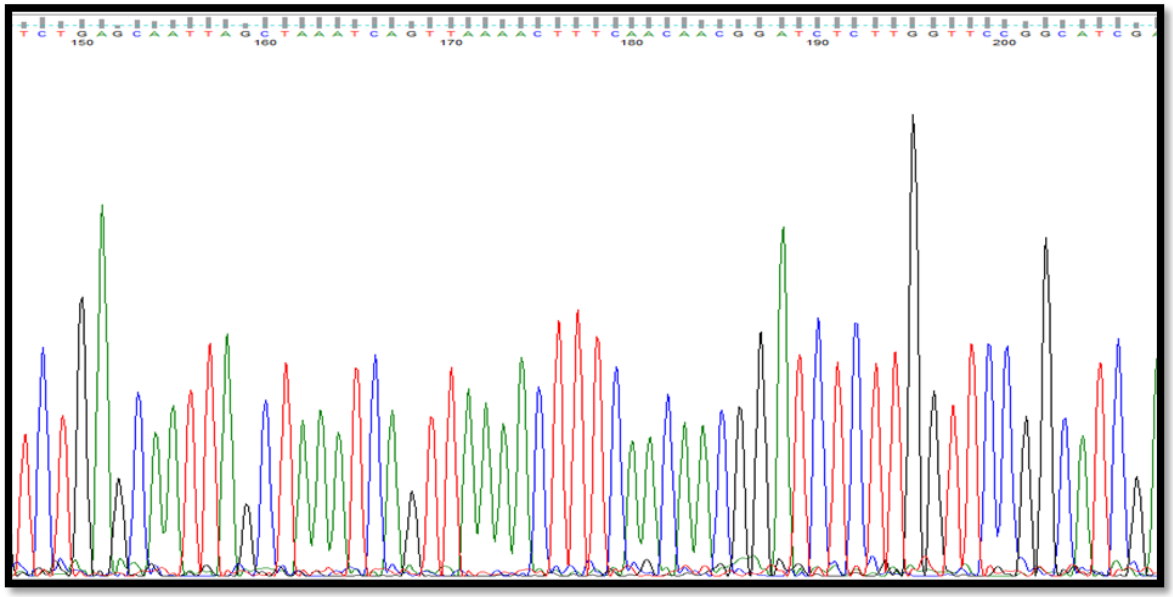
- ***Fungal endophyte***



Fungal endophyte culture-collection STRI:ICBG-Panama:TK1525 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
Sequence ID: gb|KF436280.1|Length: 1097Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
926 bits(1026)	0.0	516/518(99%)	0/518(0%)	Plus/Plus

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Query  1    GTTTATTGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGGCTTCTGCCC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  40   GTTTATTGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGCTTCTGCCC  99

Query  61   TCTGGCCCGCGCCCGCCGAAGACACCATTGAACACTGTCTGAAGATTGCAGTCTGAGCAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  100  TCTGGCCCGCGCCCGCCGAAGACACCATTGAACACTGTCTGAAGATTGCAGTCTGAGCAA  159

Query  121  TTAGCTAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAA  180
            |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  160  TTAGCTAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAA  219

Query  181  CGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  220  CGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGA  279

Query  241  ACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  280  ACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCC  339

Query  301  TCAAGCACGGCTTGTGTGTTGGGCCCCGTCCTCCTTCCCGGGGGACGGGCCCGAAAGGCA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  340  TCAAGCACGGCTTGTGTGTTGGGCCCCGTCCTCCTTCCCGGGGGACGGGCCCGAAAGGCA  399

Query  361  GCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTTGTAGGCC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  400  GCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTTGTAGGCC  459

Query  421  CGGCCGGCGCTTGCCGACAACCATCAATCTTTTTTCAGGTTGACCTCGGATCAGGTAGGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  460  CGGCCGGCGCTTGCCGACAACCATCAATCTTTTTTCAGGTTGACCTCGGATCAGGTAGGG  519

Query  481  ATACCCGCTGAACTTAAGCATATCAATAAGCGGAAGAA  518
            |||||||||||||||||||||||||||||||| |||
Sbjct  520  ATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  557
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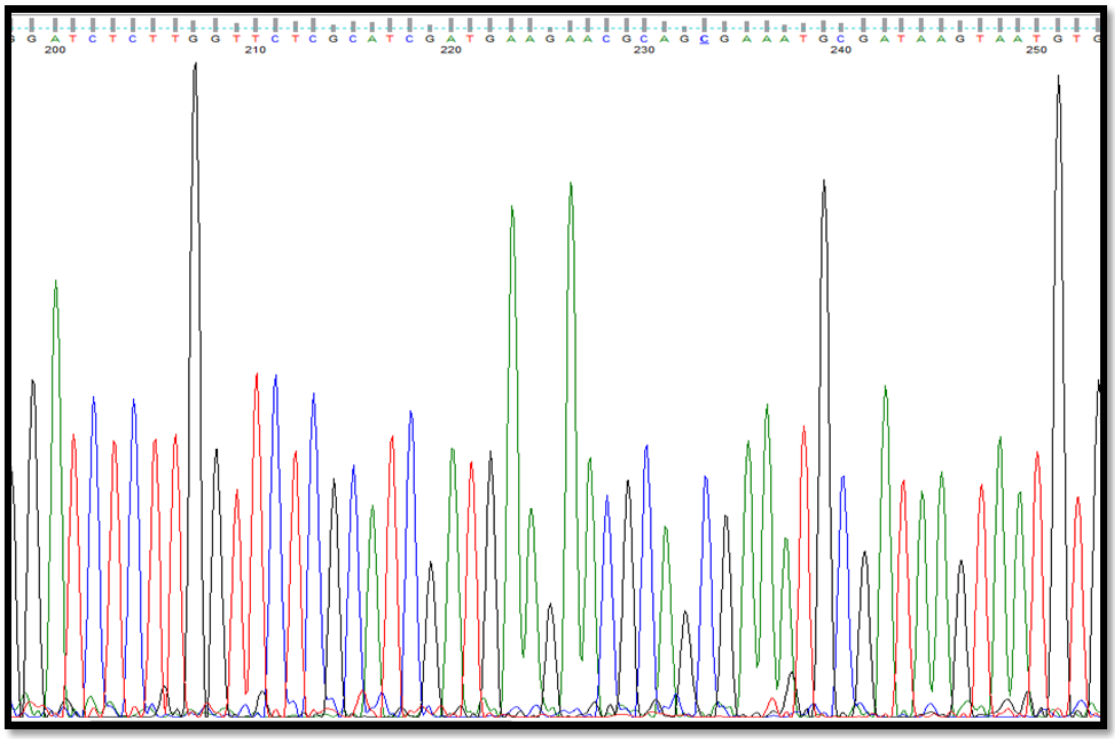
- ***Aureobasidium pullulans***



Aureobasidium pullulans strain Y11 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

Sequence ID: gb|KC897669.1|Length: 586Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
928 bits(1028)	0.0	514/514(100%)	0/514(0%)	Plus/Plus

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Query  1    GTTGTTAAAACTACCTTGTTGCTTTGGCGGGACCGCTCGGTCTCGAGCCGCTGGGGATTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  72   GTTGTTAAAACTACCTTGTTGCTTTGGCGGGACCGCTCGGTCTCGAGCCGCTGGGGATTC  131

Query  61   GTCCCAGGCGAGCGCCCGCCAGAGTTAAACCAAACTCTTGTTATTTAACCGGTCGTCTGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  132  GTCCCAGGCGAGCGCCCGCCAGAGTTAAACCAAACTCTTGTTATTTAACCGGTCGTCTGA  191

Query  121  GTTAAAATTTTGAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  192  GTTAAAATTTTGAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATG  251

Query  181  AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  252  AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC  311

Query  241  TTTGAACGCACATTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTCGAGCGTCATTAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  312  TTTGAACGCACATTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTCGAGCGTCATTAC  371

Query  301  ACCACTCAAGCTATGCTTGGTATTGGGCGTCGTCCTTAGTTGGGCGCGCCTTAAAGACCT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  372  ACCACTCAAGCTATGCTTGGTATTGGGCGTCGTCCTTAGTTGGGCGCGCCTTAAAGACCT  431

Query  361  CGGCGAGGCCACTCCGGCTTTAGGCGTAGTAGAATTTATTCGAACGTCTGTCAAAGGAGA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  432  CGGCGAGGCCACTCCGGCTTTAGGCGTAGTAGAATTTATTCGAACGTCTGTCAAAGGAGA  491

Query  421  GGAACTCTGCCGACTGAAACCTTTATTTTTCTAGGTTGACCTCGGATCAGGTAGGGATAC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  492  GGAACTCTGCCGACTGAAACCTTTATTTTTCTAGGTTGACCTCGGATCAGGTAGGGATAC  551

Query  481  CCGCTGAACTTAAGCATATCAATAAGCCGGAGGA  514
            ||||||||||||||||||||||||||||||||||
Sbjct  552  CCGCTGAACTTAAGCATATCAATAAGCCGGAGGA  585
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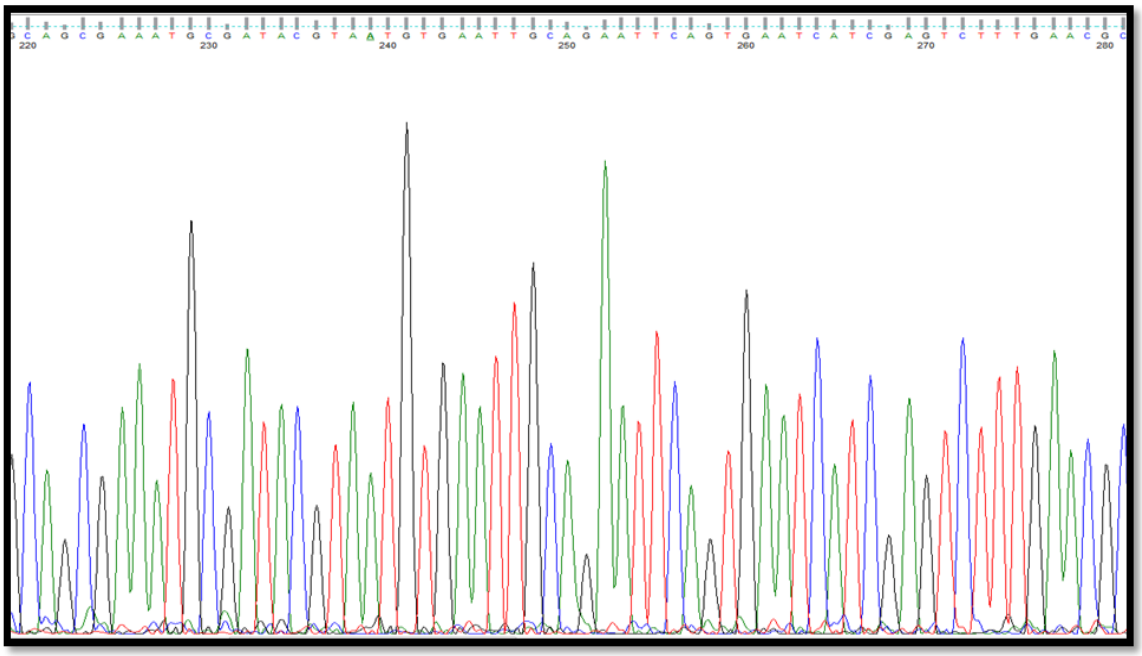
- *Alternaria alternate*



Alternaria alternata strain DHMJ19 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

Sequence ID: gb|JN986771.1|Length: 546Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
893 bits(990)	0.0	500/502(99%)	1/502(0%)	Plus/Plus

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Query  1    GTTGCTTCGGCGAGCCTGCCTTCGGGCTGCCGGGGGGCATCTGCCCCCGGGTCCGCGCTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  46   GTTGCTTCGGCGAGCCTGCCTTCGGGCTGCCGGGGGGCATCTGCCCCCGGGTCCGCGCTC  105

Query  61   GCCGGAGACACCTTGAACTCTGTCTGAAGATTGTAGTCTGAGACAAAATATAAATTATTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106  GCCGGAGACACCTTGAACTCTGTCTGAAGATTGTAGTCTGAGACAAAATATAAATTATTT  165

Query  121  AAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166  AAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGA  225

Query  181  TACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226  TACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCC  285

Query  241  TCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286  TCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGT  345

Query  301  GTGTTGGGCTCCGTCCTCCTTCTGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346  GTGTTGGGCTCCGTCCTCCTTCTGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGT  405

Query  361  CCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGACTGGCCGGCGCCTGCCG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406  CCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGACTGGCCGGCGCCTGCCG  465

Query  421  ATCAACCAAACTTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466  ATCAACCAAACTTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAA  525

Query  481  GCATATCAATAGCCGGAAGGAA  502
            ||||||||| |||||| |||||
Sbjct  526  GCATATCAAAAGCCGG-AGGAA  546
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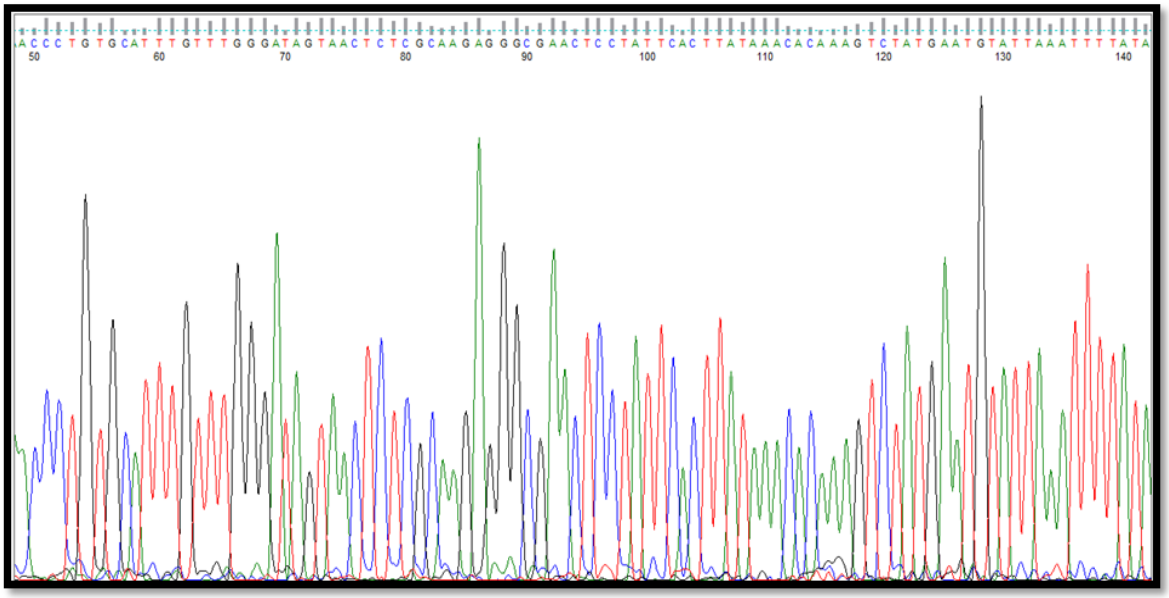
- ***Rhodotorula mucilaginosa***



Rhodotorula mucilaginosa strain UIMC35 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

Sequence ID: gb|KC816558.1|Length: 607Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
967 bits(1072)	0.0	536/536(100%)	0/536(0%)	Plus/Plus

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Query  1    CCCTGTGCATTTGTTTGGGATAGTAACTCTCGCAAGAGGGCGAACTCCTATTCACTTATA  60
Sbjct  72   CCCTGTGCATTTGTTTGGGATAGTAACTCTCGCAAGAGGGCGAACTCCTATTCACTTATA  131

Query  61   AACACAAAGTCTATGAATGTATTAAATTTTATAACAAAATAAAACTTTCAACAACGGATC  120
Sbjct  132  AACACAAAGTCTATGAATGTATTAAATTTTATAACAAAATAAAACTTTCAACAACGGATC  191

Query  121  TCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA  180
Sbjct  192  TCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA  251

Query  181  ATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCATGGTATTCCGTGGAGCAT  240
Sbjct  252  ATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCATGGTATTCCGTGGAGCAT  311

Query  241  GCCTGTTTGAGTGTCATGAATACTTCAACCCTCCTCTTTCTTAATGATTGAAGAGGTGTT  300
Sbjct  312  GCCTGTTTGAGTGTCATGAATACTTCAACCCTCCTCTTTCTTAATGATTGAAGAGGTGTT  371

Query  301  TGGTTTCTGAGCGCTGCTGGCCTTTAGGGTCTAGCTCGTTCGTAATGCATTAGCATCCGC  360
Sbjct  372  TGGTTTCTGAGCGCTGCTGGCCTTTAGGGTCTAGCTCGTTCGTAATGCATTAGCATCCGC  431

Query  361  AATCGAACTTCGGATTGACTTGGCGTAATAGACTATTCGCTGAGGAATTCTAGTCTTCGG  420
Sbjct  432  AATCGAACTTCGGATTGACTTGGCGTAATAGACTATTCGCTGAGGAATTCTAGTCTTCGG  491

Query  421  ACTAGAGCCGGGTTGGGTTAAAGGAAGCTTCTAATCAGAATGTCTACATTTTAAGATTAG  480
Sbjct  492  ACTAGAGCCGGGTTGGGTTAAAGGAAGCTTCTAATCAGAATGTCTACATTTTAAGATTAG  551

Query  481  ATCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  536
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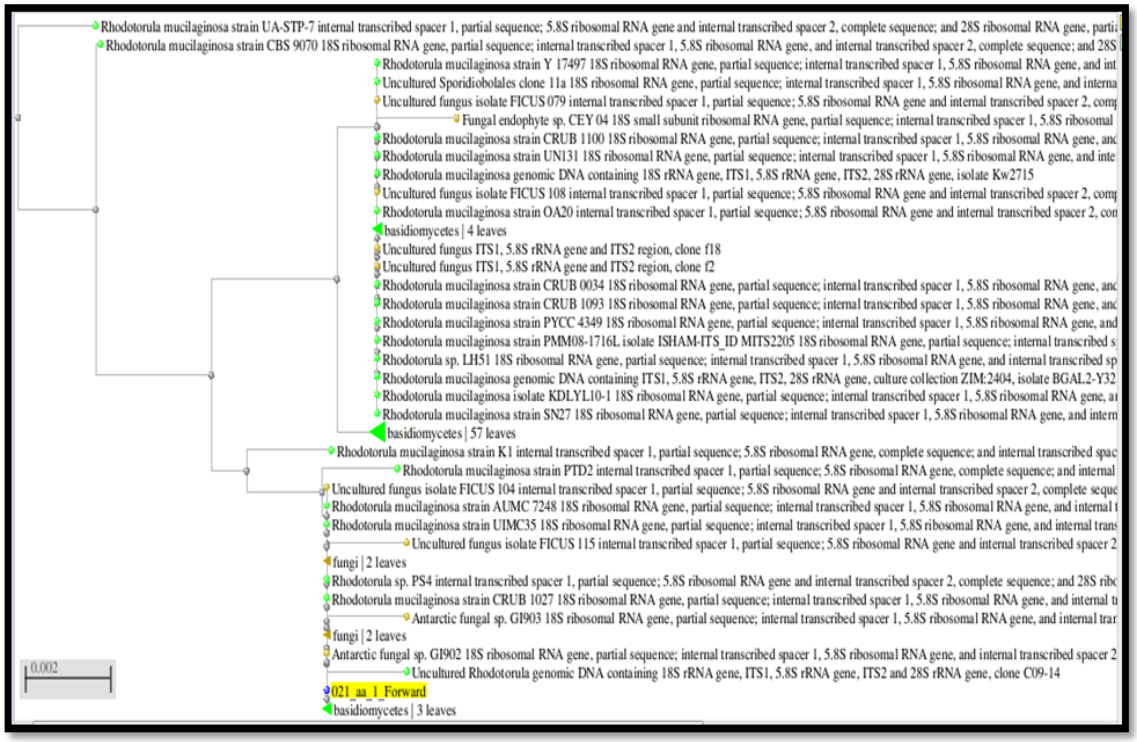
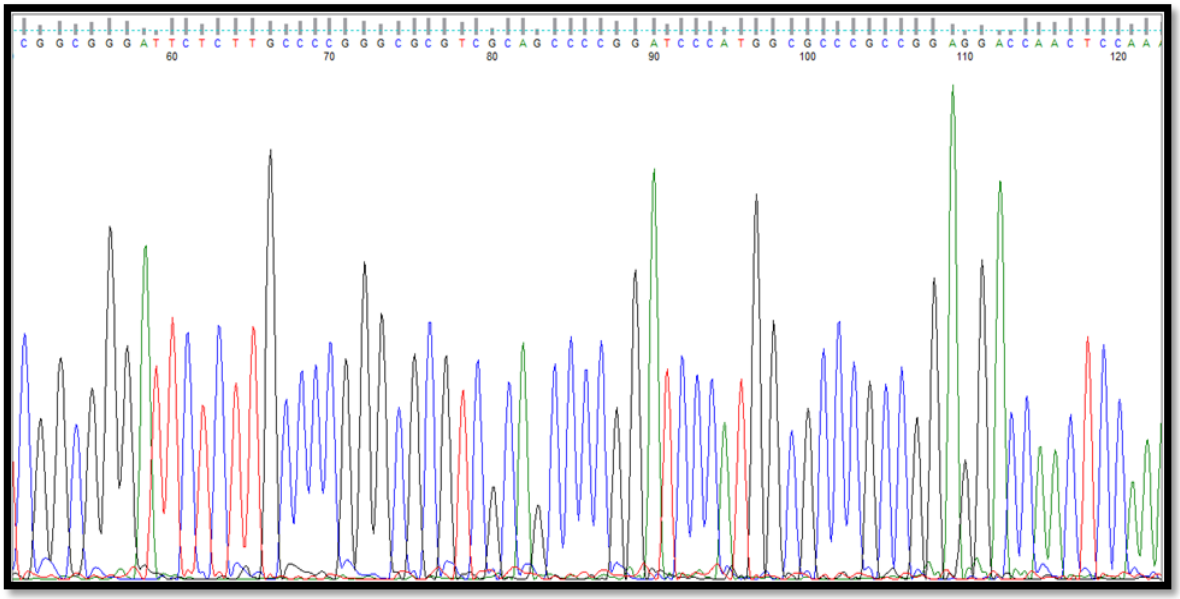


- ***Trichoderma reesei***



Trichoderma reesei strain S4-P-2-3 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

Sequence ID: gb|KP216890.1|Length: 591Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
663 bits(734)	0.0	367/367(100%)	0/367(0%)	Plus/Plus

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Query  1    GCCTCGGCGGGATTCTCTTGCCCCGGGCGCGTCGCAGCCCCGGATCCCATGGCGCCCGCC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  30   GCCTCGGCGGGATTCTCTTGCCCCGGGCGCGTCGCAGCCCCGGATCCCATGGCGCCCGCC  89

Query  61   GGAGGACCAACTCCAAACTCTTTTTTCTCTCCGTCGCGGCTCCCGTCGCGGCTCTGTTTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  90   GGAGGACCAACTCCAAACTCTTTTTTCTCTCCGTCGCGGCTCCCGTCGCGGCTCTGTTTT  149

Query  121  ATTTTTGCTCTGAGCCTTTCTCGGCGACCCTAGCGGGCGTCTCGAAAATGAATCAAAACT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150  ATTTTTGCTCTGAGCCTTTCTCGGCGACCCTAGCGGGCGTCTCGAAAATGAATCAAAACT  209

Query  181  TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  210  TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA  269

Query  241  ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT  300
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Sbjct  270  ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT  329

Query  301  ATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGGTCG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  330  ATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGGTCG  389

Query  361  GCGTTGG  367
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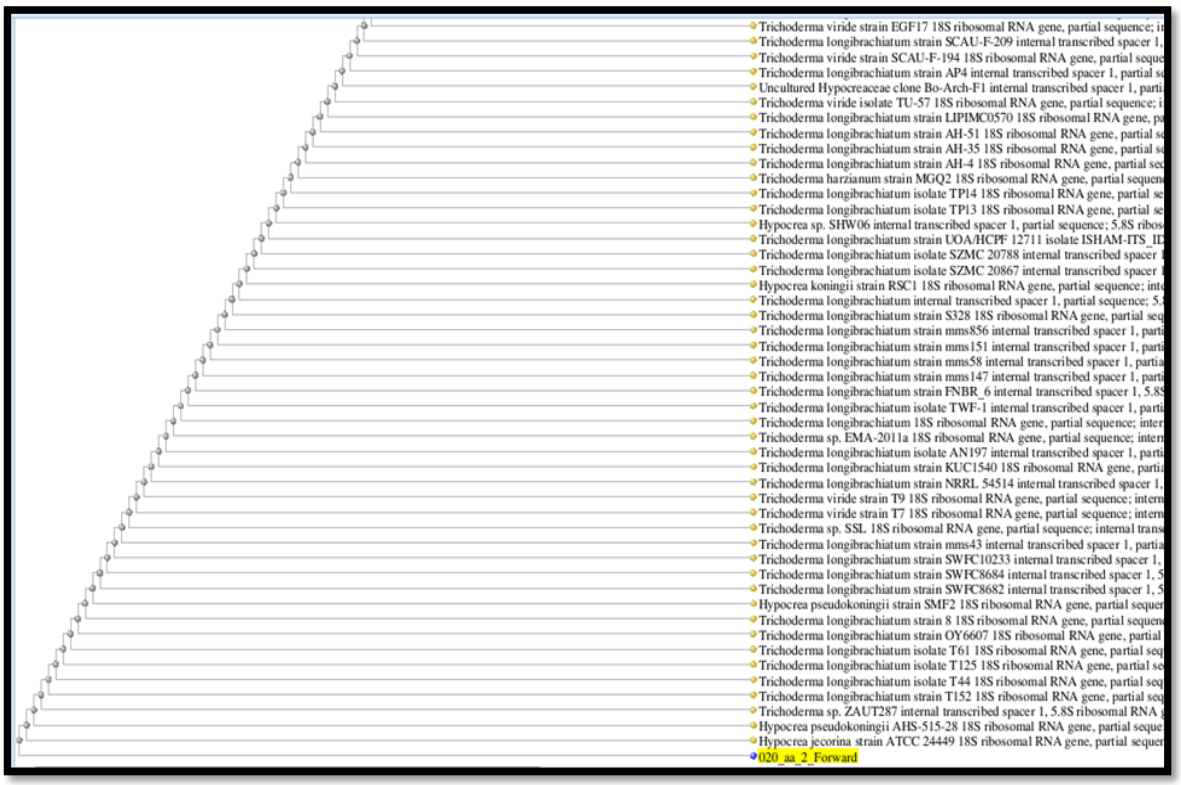
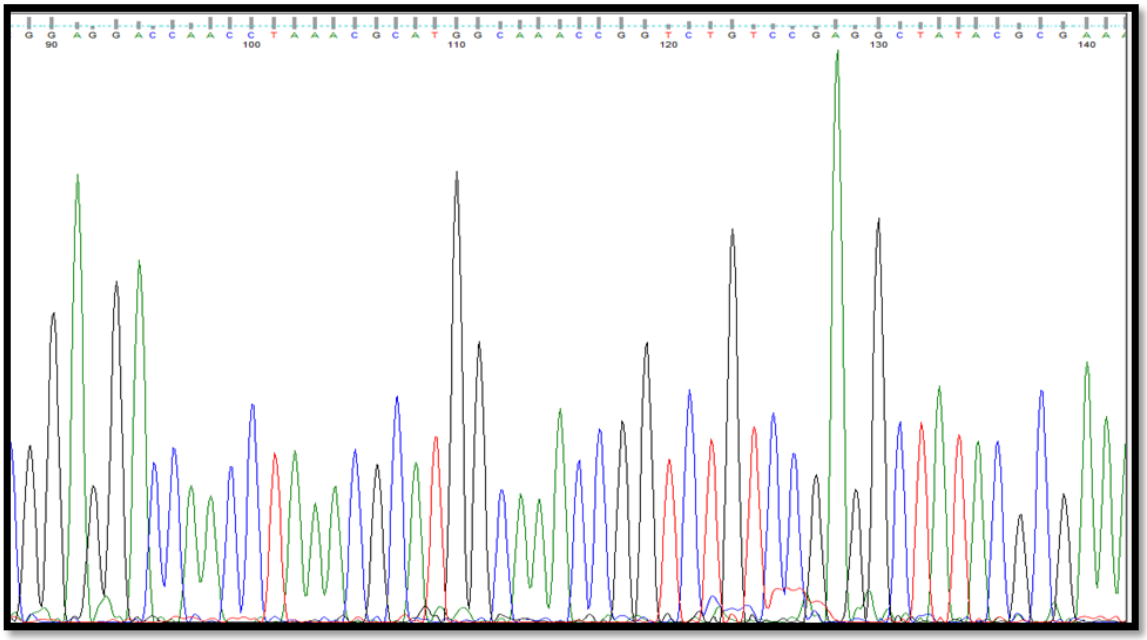


- ***Arthrographis kalrae***



Arthrographis kalrae strain FMR 12123 isolate ISHAM-ITS_ID MITS98 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

Sequence ID: gb|KP131545.1|Length: 534Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
513 bits(568)	7e-142	284/284(100%)	0/284(0%)	Plus/Plus

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Query  1    GCAGTTGCTTCGGCGGCGGCGCCCTCGCGGGCCCCCGCCGGAGGACCAACCTAAACGCAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  80   GCAGTTGCTTCGGCGGCGGCGCCCTCGCGGGCCCCCGCCGGAGGACCAACCTAAACGCAT  139

Query  61   GGCAAACCGGTCTGTCCGAGGCTATACGCGAAATCAGTCAAAACTTTCAACAACGGATCT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  140  GGCAAACCGGTCTGTCCGAGGCTATACGCGAAATCAGTCAAAACTTTCAACAACGGATCT  199

Query  121  CTTGGTTCGGGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  200  CTTGGTTCGGGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAA  259

Query  181  TTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGTCGGCATTCCGACGGGCACG  240
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Sbjct  260  TTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGTCGGCATTCCGACGGGCACG  319

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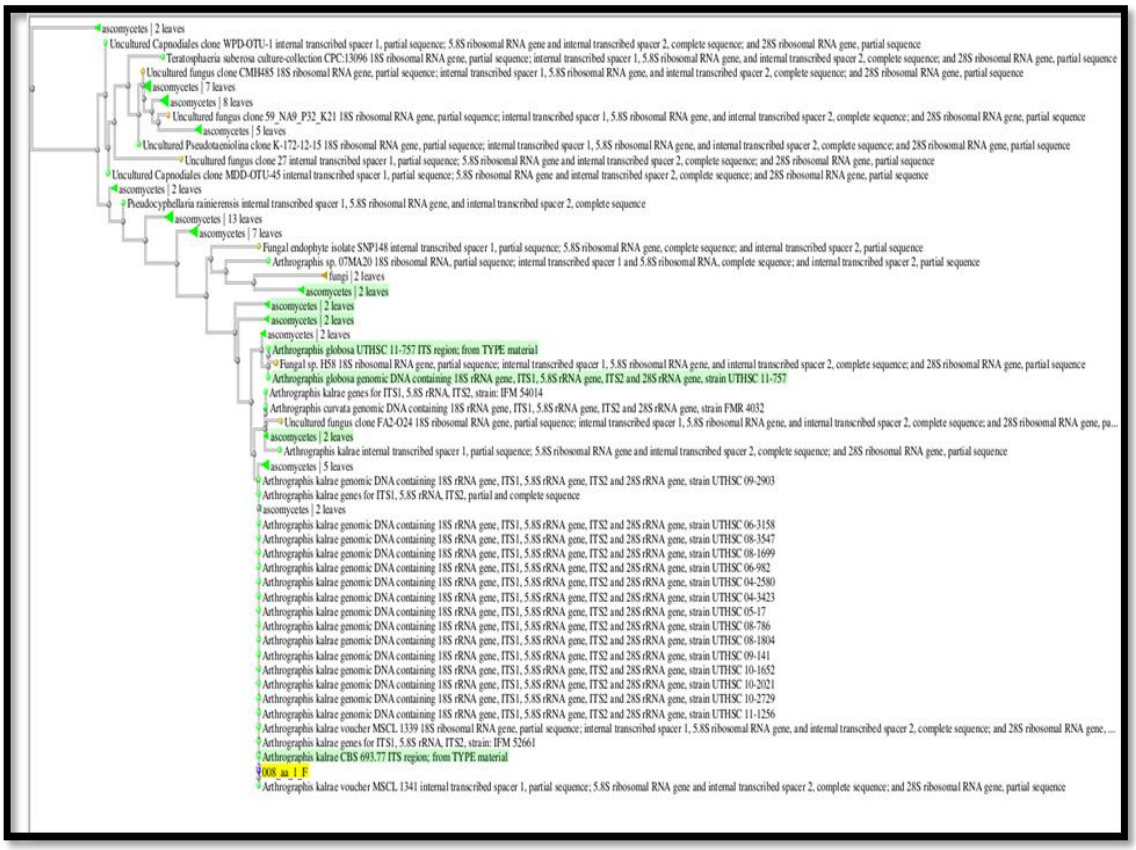
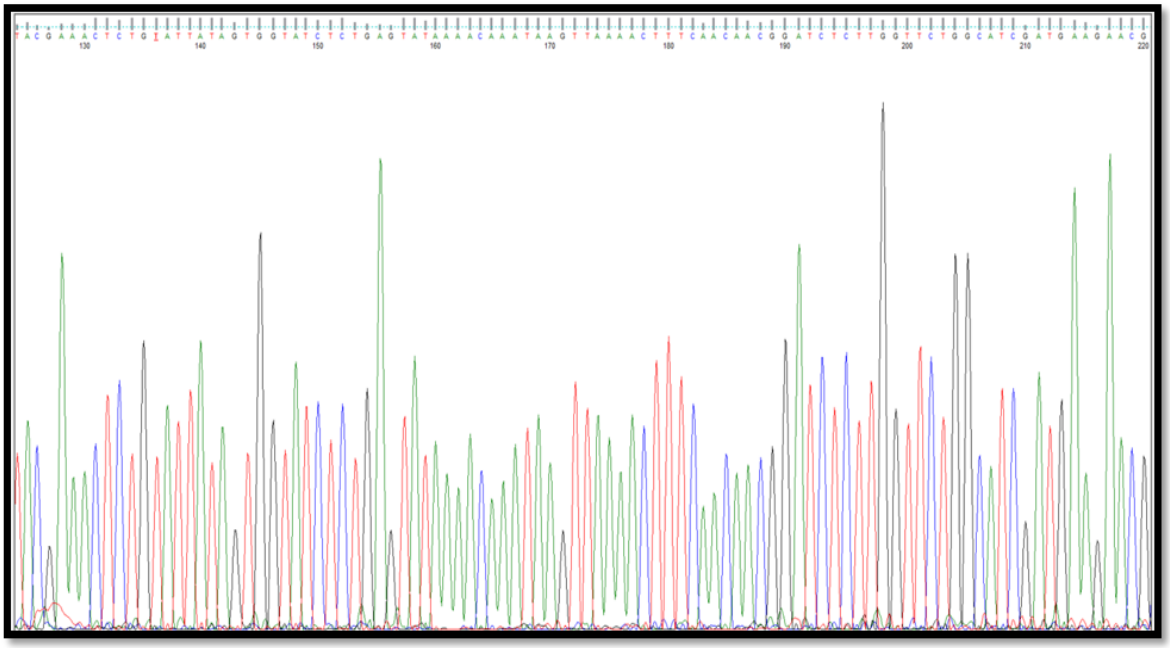


- ***Lecythophora hoffmannii***



Lecythophora sp. KAUH21 genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, clone 21

Sequence ID: emb|LN827695.1|Length: 561Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
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Sbjct  178  AAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGA  237

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Sbjct  238  TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC  297

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Sbjct  298  GCTAGTACTCTAGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGG  357

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Sbjct  358  TGTTGGGGCTCTACGTCTGACGTAGGCCCTGAAAGGAAGTGGCGGGCTCGCTACAACTCC  417

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Query  421  CATCTTTAACCAAGGTTGACCTCGGATCAGG  451
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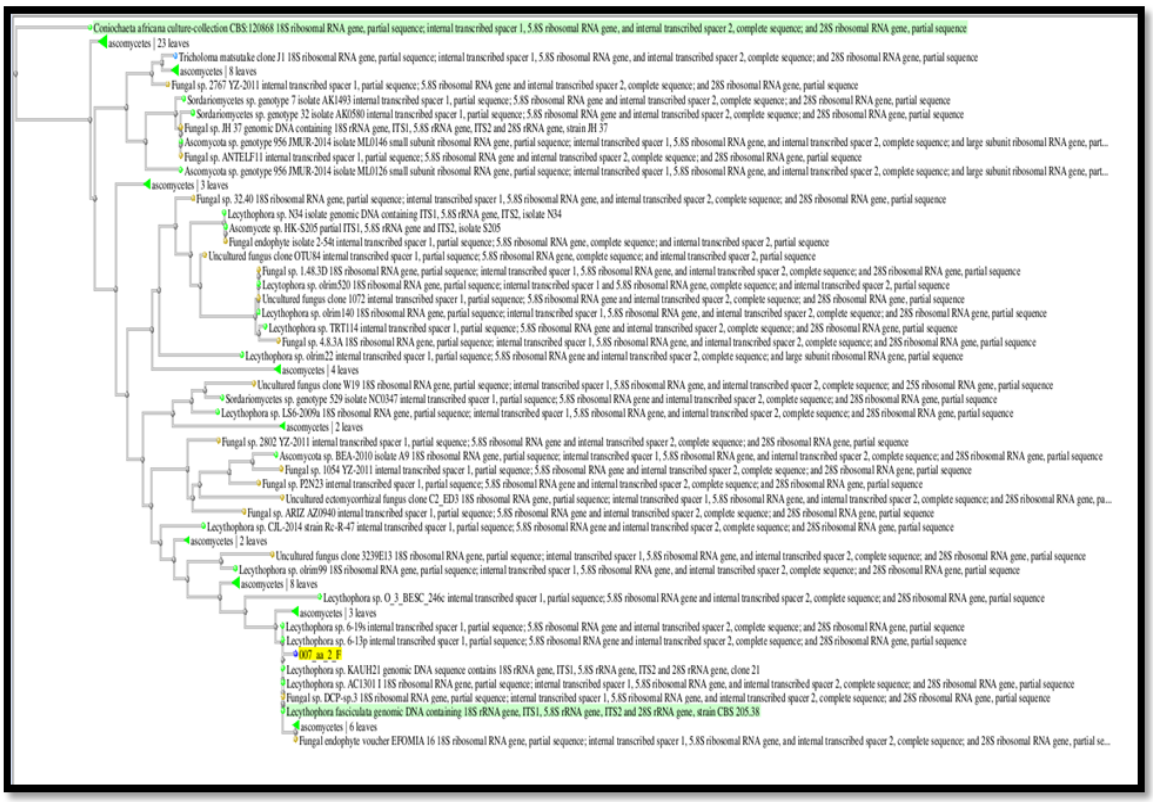
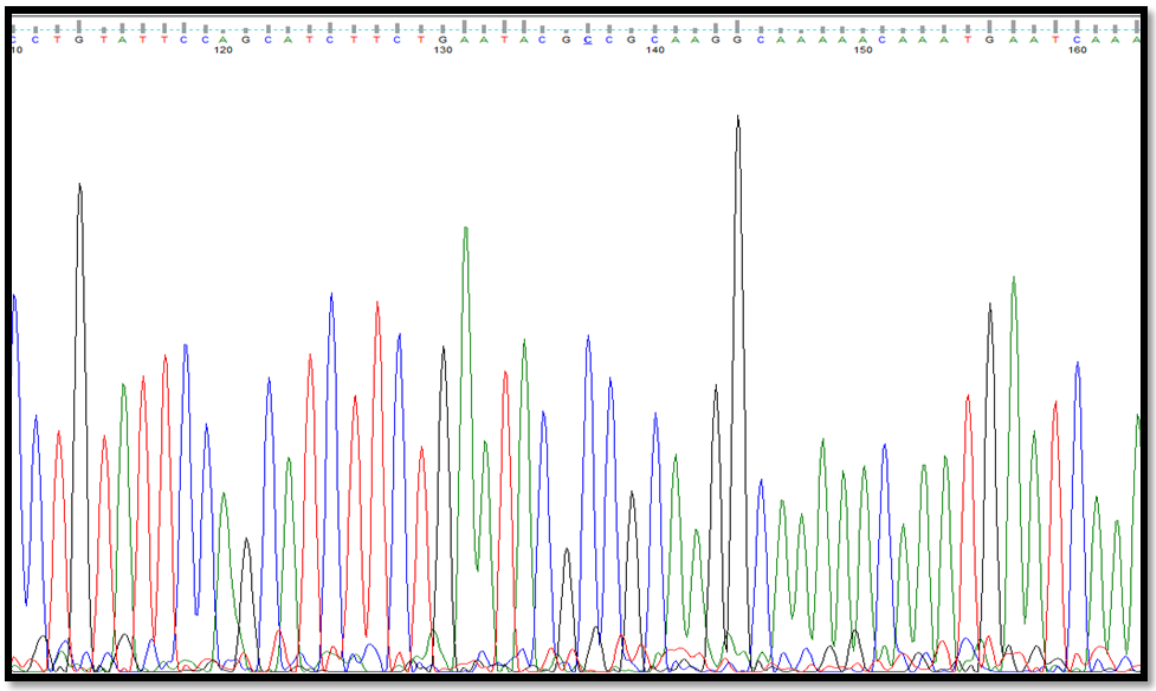


- ***Beauveria bassiana***



Beauveria bassiana isolate DAOM210087 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

Sequence ID: gb|EU334678.1|Length: 6635Number of Matches: 1

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Sbjct  2274  CCTGTATTCCAGCATCTTCTGAATACGCCGCAAGGCAAAAACAAATGAATCAAAACTTTC  2333

Query  121   AACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG  180
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Sbjct  2334  AACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG  2393

Query  181   TGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGCCAGCATT  240
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Sbjct  2394  TGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATT  2453

Query  241   CTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCGACCTCCCCTTGGGGAGGTCGG  300
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Query  301   CGTTGGGGACCGGCAGCACACCGCCGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGA  360
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Sbjct  2514  CGTTGGGGACCGGCAGCACACCGCCGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGA  2573

Query  361   CCTCTGCGTAGTAATACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTAAAACACCC  420
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Sbjct  2574  CCTCTGCGTAGTAATACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTAAAACACCC  2633

Query  421   AACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAAT  480
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Sbjct  2634  AACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAAT  2693

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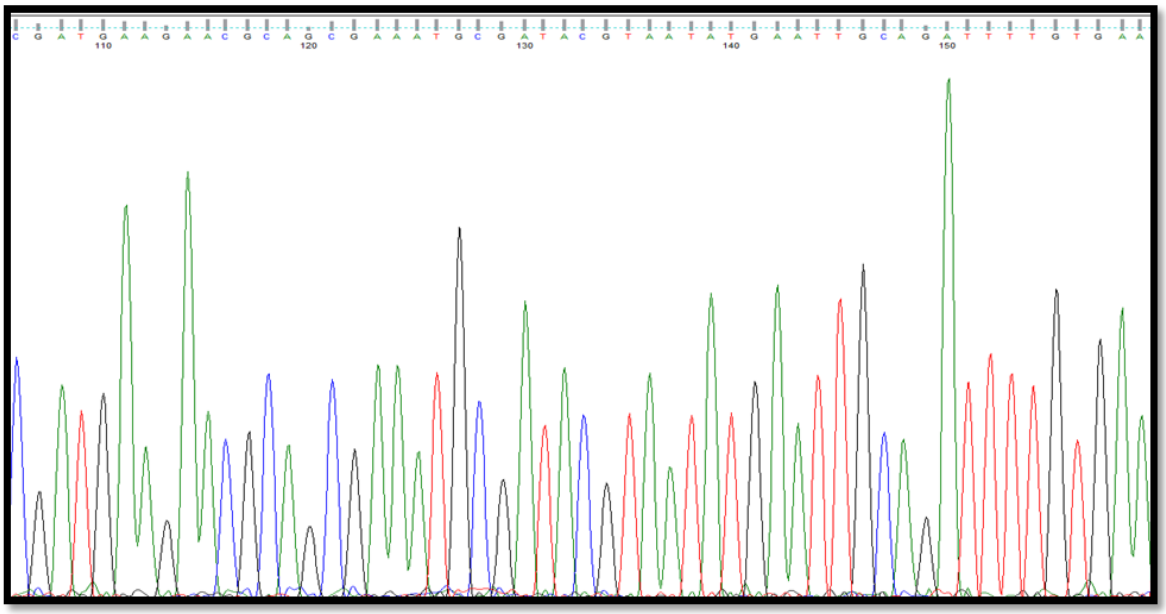
- ***Candida catenulate***



Candida catenulata strain PMM08-3259N3L isolate ISHAM-ITS_ID MITS540 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

Sequence ID: gb|KP131684.1|Length: 769Number of Matches: 1

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Sbjct  283  TCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATTTTGTGAA  342

Query  121  TCATCGAATCTTTGAACGCACATTGCGCCCGTGGGTATTCCCGCGGGCATGCGTGTGTGA  180
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Query  181  GCGGTTATCTCCCTCGCAGGTAATGGACATACGGCGTCAGCTGTTCCTCCAAAAAGTGAT  240
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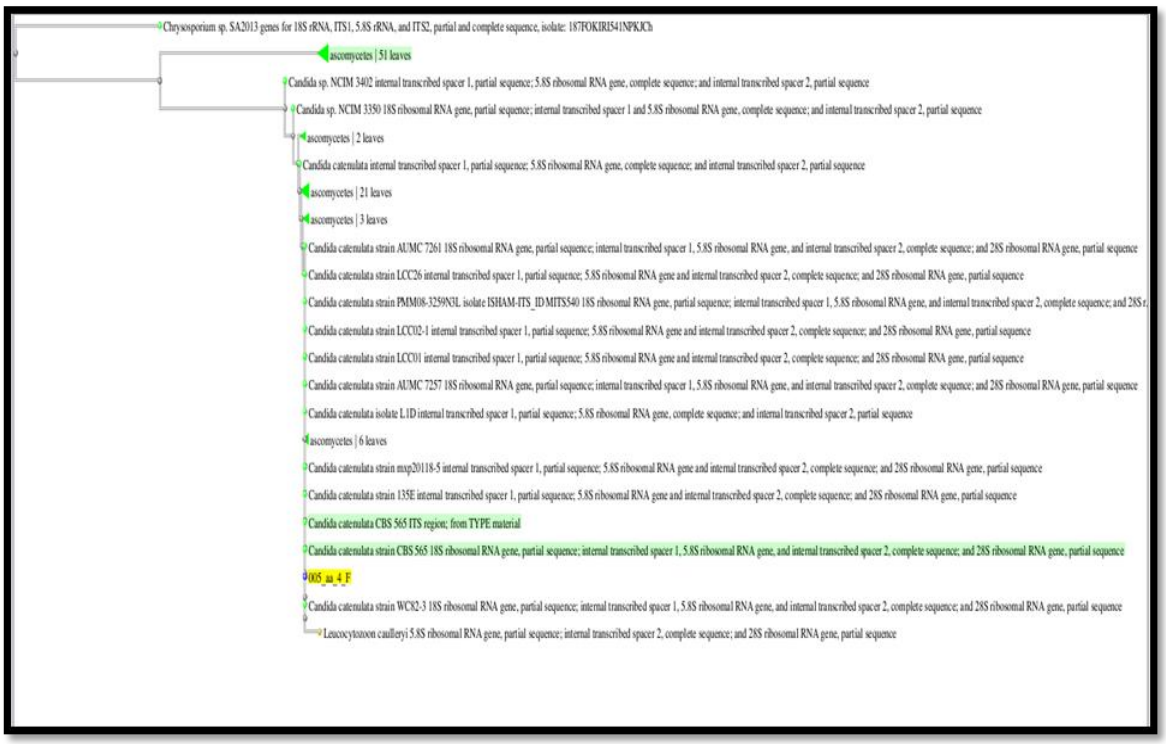
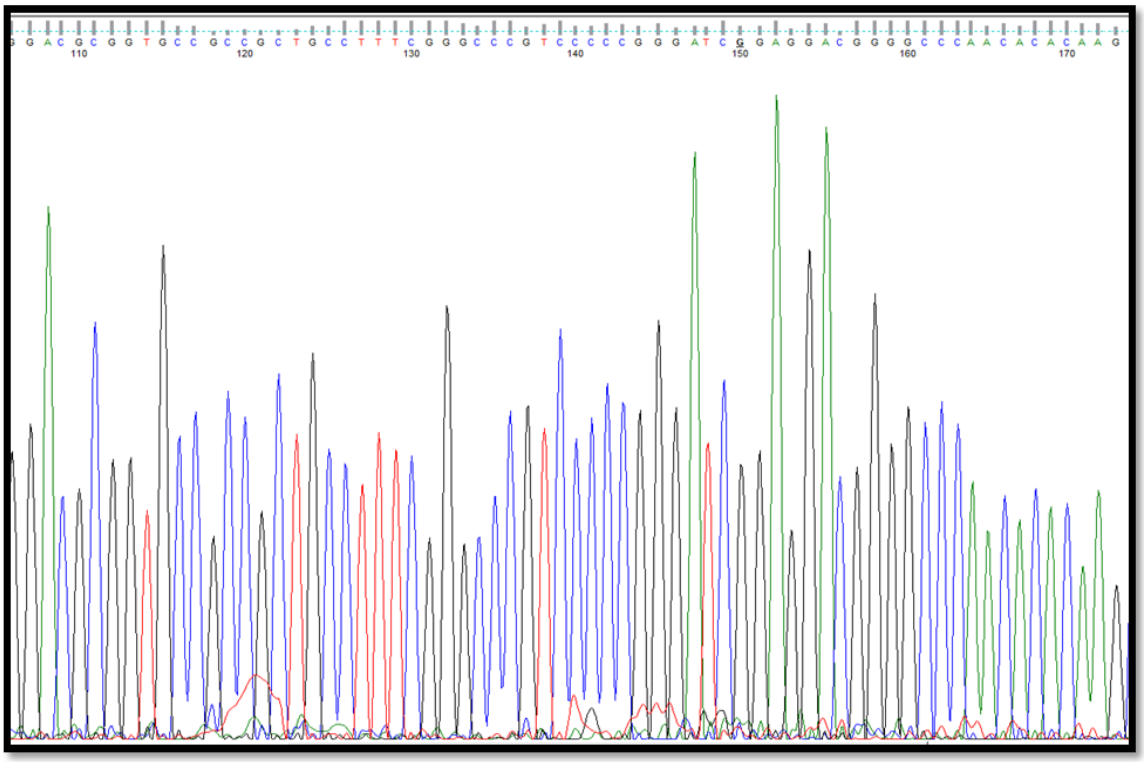


- *Penicillium flavigenum*



Penicillium flavigenum voucher E.2.7 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

Sequence ID: gb|KR261456.1|Length: 536Number of Matches: 1

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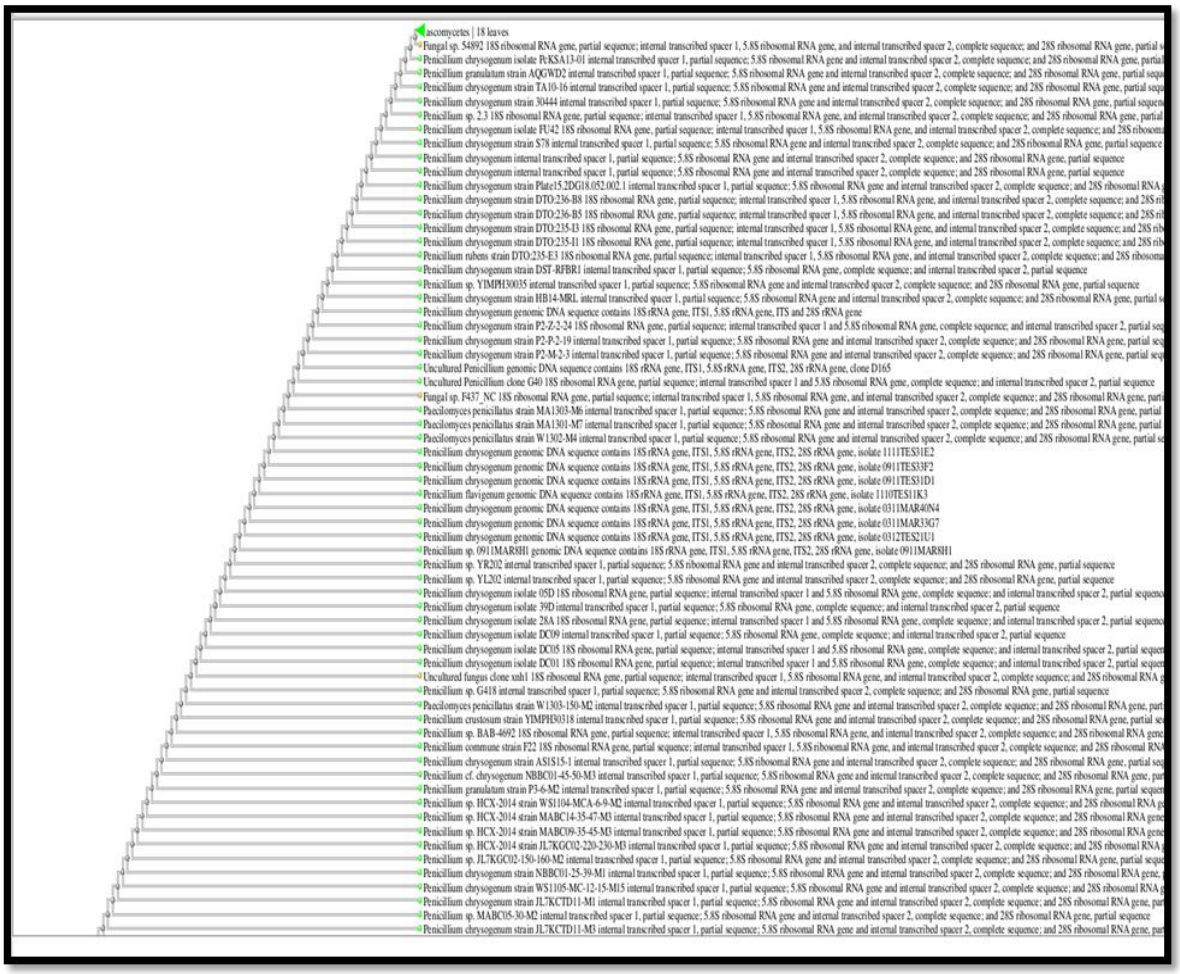
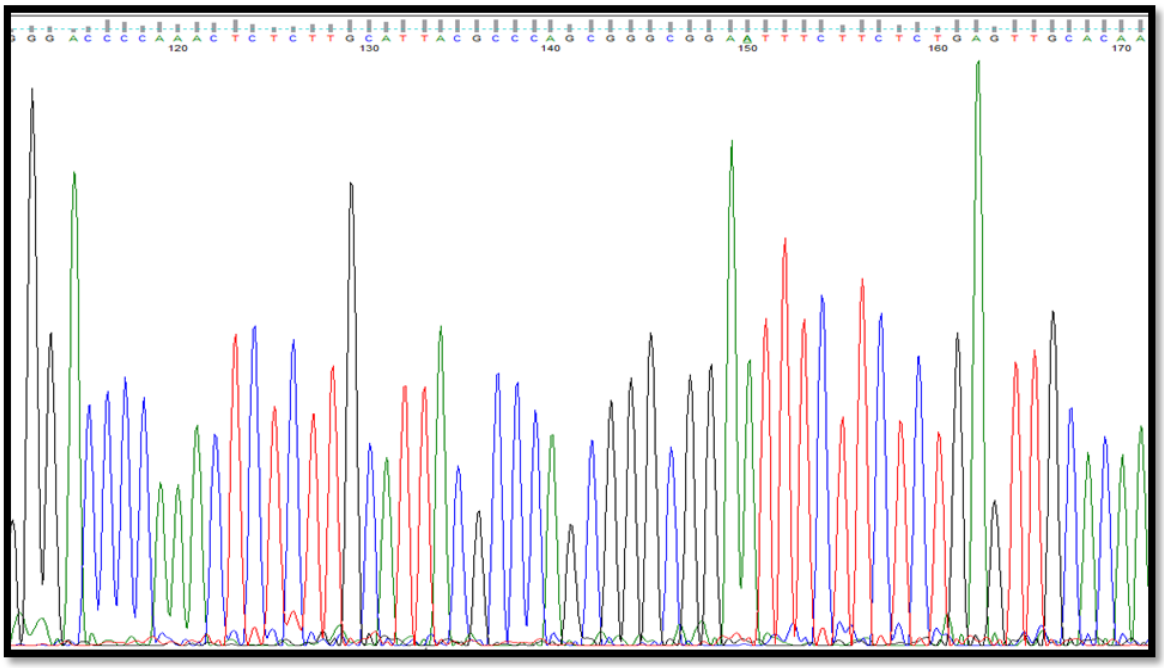


- ***Purpureocillium lilacinum***



Purpureocillium lilacinum strain NPF-4 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

Sequence ID: gb|KP308838.1|Length: 565Number of Matches: 1

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Sbjct  87   CGGACCCAGGCGCCCGCCGCAGGGACCCCAAACTCTCTTGCATTACGCCCAGCGGGCGGA  146

Query  121  ATTTCTTCTCTGAGTTGCACAAGCAAAAACAAATGAATCAAAACTTTCAACAACGGATCT  180
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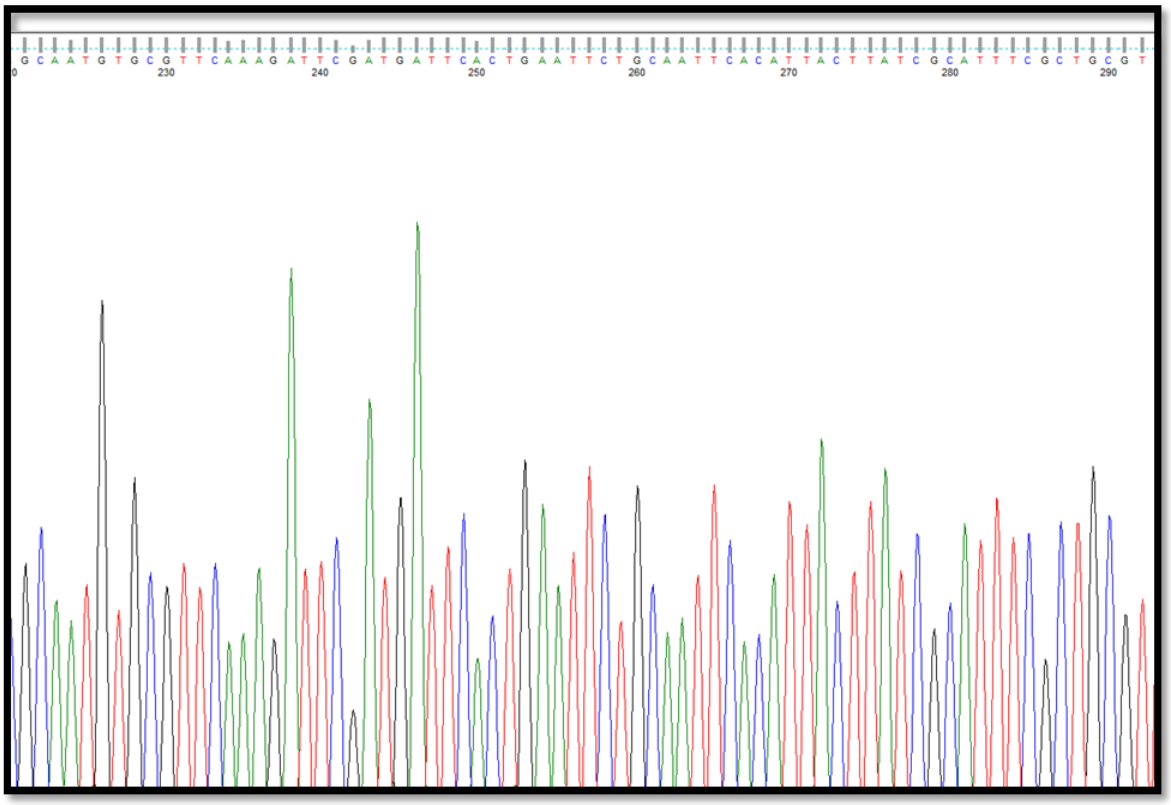
- ***Malbranchea cinnamomea***



Malbranchea cinnamomea strain HSAUP0380003 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

Sequence ID: gb|GU966515.1|Length: 573Number of Matches: 1

Range 1: 1 to 502 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
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Sbjct  82   AAGCAACGGTTTAGGTAACGTTCACAATGGTGTAGGGAGTTTTGCAACTCTGTAATGATC  23

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The Role of *Candida* and Other Yeasts in the Environment Notably in Relation to Biogeochemical Cycles

The University Of Sheffield.
Department of Molecular Biology and Biotechnology

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وزارة الصحة
Ministry of Health

Abstract

Microorganisms play a key role in the functioning of the environment in relation to the biogeochemical cycles and also play a crucial role in transforming and recycling essential elements; as a result, they make an important contribution to biogeochemical cycles and biotechnological processes. Here, a study was made of the role of isolated yeasts in aspects of the Nitrogen cycle, i.e. specifically the hydrolysis of Urea to Ammonium and Oxidation of Ammonium to Nitrate. Other microbial transformations studied included Phosphate solubilization and the oxidation of elemental Sulphur to Sulphate.

Introduction

Several studies show that microorganisms play an important role in the functioning of the environment in relation to the major biogeochemical cycles (Bielnska-Warezak and Nowicki, 2005). The role of yeasts in environmental functioning has however, been largely overlooked. Yeasts exhibit specific associations with certain insects (Suh *et al.*, 2003) and some *Candida sp.* Have the ability to form biofilms on the surfaces of both organic and inorganic materials and are found in a variety of the many different environments particularly in soils, on leaves and flowers, in water, soil and are also widely associated with dung. Wyatt *et al.* (1975) showed that *Candida* could be isolated from most crops and from healthy birds. Kirk and Bartlett (1986) and Okamoto *et al.* (1988) isolated *Candida sp.* from milk samples taken from cows with signs of mastitis and this yeast has also been found in mastitis-free cattle. Clark (1960) found that bovine mycotic mastitis may occur by infection via licking or suckling of the udder quarters by lactating calves or through contamination of the udder with the animal's own dung. Williams *et al.* (1977) also isolated *Candida sp.* as the a etiological agent of abortion in cattle.

Aims of the Project

The aim of this project is to determine yeast diversity in the environment using molecular identification techniques, notably 18S rRNA. Classical microbiology and molecular techniques were employed to identify and characterize yeasts and other fungi isolated from a number of environments. The ability of these isolates to participate in mineral transformations was also determined.

Methods

A variety of yeasts, isolated from cattle dung (i.e. cow dung) were identified using molecular identification techniques based on DNA extraction, PCR amplification and determination of the sequences 18S rRNA genes.
Two yeasts were isolated, namely *Candida rugosa*, and *Candida inconspicua* and these isolates were studied in relation to their poetical role in mineral cycling, i.e.:
1- Determination of the hydrolysis of Urea to Ammonium by yeasts.
2- Determination of the Oxidation of Ammonium to Nitrate by yeasts.
3- Determination the Oxidation of Sulphur by yeasts.
4- Determination the Solubilization of Phosphate by yeasts.

Results

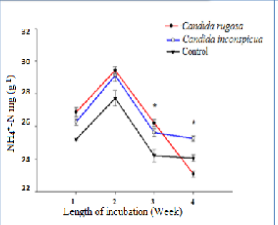

Figure 1: Ammonium production from the hydrolysis of Urea by yeasts.
Standard error (SE): * significant difference from control P<0.050.

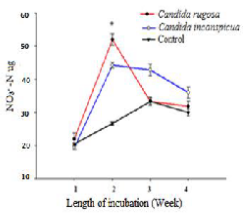

Figure 2: Nitrate production from the oxidation of Ammonium by yeasts.
Standard error (SE): * significant difference from control P<0.050.

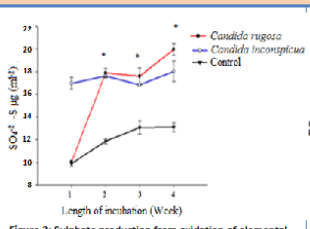

Figure 3: Sulphate production from oxidation of elemental Sulphur by yeasts
Standard error (SE): * significant difference from control P<0.050.

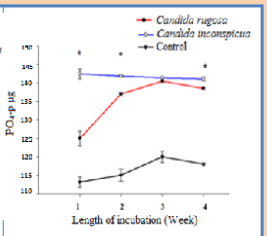

Figure 4: Phosphate solubilization by yeasts.
Standard error (SE): * significant difference from control P<0.050.

This experiments was performed in order to compare the activity of two types of yeast and the control over the four week incubation period.
Figure 1, Ammonium production from the hydrolysis of Urea; ammonium production being measured. The overall conclusion is that both yeasts hydrolyse urea to ammonium with no major differences being found in rates of hydrolysis between *Candida rugosa* and *Candida inconspicua*.
Figure 2, Nitrate production from the oxidation of Ammonium; nitrification, i.e. the production of nitrate being measured. The overall conclusion is that both yeasts oxidize ammonium with no major differences being found in rates of oxidation between *Candida rugosa* and *Candida inconspicua.*
Figure 3, Sulphate production from oxidation of elemental Sulphur; Sulphur oxidation i.e. the production of sulphate being measured. The overall conclusion is that both yeasts oxidized Sulphur with no major differences being found in rates of oxidation between *Candida rugosa* and *Candida inconspicua*.
Figure 4, Phosphate solubilization; was performed in order determine the solubilization of calcium phosphate (CaPO4), by the yeasty. The overall conclusion is that both yeasts solubilized insoluble phosphate with no major differences being found in rates of soluibilzation between *Candida rugosa* and *Candida inconspicua*.

Conclusion

Two yeasts species were isolated from cattle dung and identified using 18S rRNA as *Candida rugosa* and *Candida inconspicua*. Both species were equally efficient at a) Oxidizing Ammonium to Nitrate b) Sulphur to Sulphate, c) Hydrolyzing Urea to ammonium, and d) Solubilizing insoluble phosphate. As a result, we suggest that these yeasts can play a major role in the N and S cycle, both in cow dung and in adjacent soils.

Future Work

- Relatively little is known about the role of yeasts in the hospitals environments, natural environments and the built environment, also the factors which influence their survival.
- In future I will determine the factors which influence the survival of *Candida* in the environments (e.g. on ceramic tiles, in washrooms, in toilets, sinks, medical equipment and hand dryers) notably in relation to the survival of pathogenic yeasts in hospitals, due to the hospital environment is potential reservoir of pathogens and other medical environments.

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