Molecular Identification and Characterisation of Extremophilic and Pathogenic Microorganisms from Water Samples Collected in the UK and Saudi Arabia

By

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Dedication

To the home of knowledge, culture and civilization
To Al-Hassa
I dedicate this humble work
To the memory of my father Sheikh Hussain
Certainly, you are proud of me, and I do hope that we meet in heaven, with Allah's willing. To my great Mum.
To my beloved wife "Ibtihaj" and my sons Mohammed, Alhassan and the lovely baby Nasser and sweet daughter Fatima
To my brothers and sisters

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Abstract

Water samples were collected from the rivers Lathkill and Bradford, Derbyshire UK and from the Al-Asfar lake and irrigation channels, Hassa, Saudi Arabia. The Derbyshire samples were incubated in rich growth medium at pH 10 and two alkaliphilic/alkalitolerant strains were isolated. Molecular identification techniques based on 16S rRNA gene sequencing suggested that both strains belonged to the *Exiguobacterium* genus of Gram-positive bacteria. The Hassa samples were incubated in highly saline rich growth media (up to 4 M NaCl) and three halophilic or halotolerant strains were isolated. In this case 16S rRNA gene sequencing identified the strains as *Halomonas venusta*, *Halobacillus blutaparonensis* and *Staphylococcus warneri*.

The *Exiguobacterium* strains were further characterised with respect to antibiotic sensitivity and carbon source utilisation. The compatible solute betaine was detected in *Exiguobacterium* cells grown at pH 10; this is the first report of a compatible solute being found in *Exiguobacterium* cells.

Halobacillus blutaparonensis and *Staphylococcus warneri* were further characterised and *H. blutaparonensis* was shown to accumulate betaine which allows growth at high salinity. However, *H. blutaparonensis* was unable to synthesise the alternative compatible solute ectoine and was therefore incapable of growth at high salinity in minimal medium. *S. warneri* is an important hospital acquired pathogen and it was interesting that it was found in the Hassa water. It also accumulated betaine to allow growth at high salinities.

Two strains (*Exiguobacterium* and *S. warneri*) were chosen for experiments examining the effect of UVC light (254 nm) on cell viability, DNA structure and cell morphology. On solid medium, both strains were susceptible to UVC light with most cells killed after a few minutes exposure. In liquid cultures, *Exiguobacterium* strain was more resistant. Gel electrophoresis studies showed that the DNA was degraded by increasing exposure to UV light, but no clear effects were seen on cell morphology using electron microscopy.

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Abbreviations

bp	Base pair (s)
BSA	Bovine serum albumin
^{o}C	Centigrade
CFE	Cell free extract
Caps	3-(cyclohexylamino)-1-propanesulfonic acid
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
EB	Ethidium bromide
FAD	flavin adenine dinucleotide
g	Gram (s)
h	Hour (s)
kb	Kilobase (s)
LB	Luria-Bertani medium
M	Molar
Mes	2-(N-morpholino)ethanesulfonic acid
mg	Milligram (s)
min	Minute (s)
ml	Millilitre (s)
mM	Millimole (s)
Mops	3-(N-morpholino)propanesulfonic acid
MW	Molecular weight
NAD	Nicotinamide adenine dinucleotide (oxidised form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
OAA	Oxaloacetic acid
OD	Optical density
PCR	Polymerase chain reaction
rDNA	ribosomal DNA
RNA	Ribonucleic acid
rRNA	Ribosomal Ribonucleic acid
RNase	Ribonuclease