

**Isolation and Characterization of Extremophilic Microorganisms
from Industrial Effluents**



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BS

BIOTECHNOLOGY

BY

Muneeba Waseem Ghauri

ID

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Session: 2014-2018

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DECLARATION

I hereby Muneeba Waseem Ghauri of the degree of Bachelors of Science in Biotechnology with Student ID, 14001231001, declare in this declaration that this is my own original work titled as **“Isolation and Characterization of Extremophilic Microorganisms from Industrial Effluents.”**Appropriate references are provided where any collaboration with other researchers is involved or if any materials or methods are mentioned in this study. This work is being submitted to the University of Management and Technology in fulfillment of the requirements. No copy of this thesis has been submitted to any other university or institution for any degree or examination.

Dated: _____

Muneeba Waseem Ghauri

RESEARCH COMPLETION CERTIFICATE

This is to certify that the entitled thesis, “**Isolation and characterization of Extremophilic Microorganisms from Industrial Effluent**” submitted by **Muneeba Waseem Ghauri (14001231001)** has been accepted towards the partial fulfillment of BS degree in Biotechnology. The quantum and quality work in this thesis is adequate for the reward of the degree.

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DEDICATION

I dedicate this dissertation to my parents, due to their prayers and countless efforts made this work possible and my friends whom I had their support throughout the research work.

ABSTRACT

The aim of this study was to isolate and identify extremophiles from the industrial effluent of Pakistan. Characterization of identified extremophile using biochemical and molecular approaches, their biotechnological potential in the production of extracellular enzymes and studying the phylogenetic affiliation of the extremophilic microbes in comparison with others. The range of temperature for the growth of the isolated strain ranged from 35°C to 37°C with its optimum growth at 37°C. Physiological analysis of strain were colonies in light yellowish cream color and spherical in shape. The range of pH for the isolated strain to grow ranged from pH 9 to pH 12 with its optimum growth at 37°C. Optimum NaCl and pH concentration for the strain to grow were 5% and 9.0, respectively. The full-length 16S rRNA sequence was 92.50% homologous to that of *Alishewanella jeotgali* strain MS1 and *Alishewanella agri* strain BAB6492.

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I am thankful to Almighty Allah, the most gracious, who in His infinite mercy has guided me to complete this research work. May Peace and Blessings be upon His Prophet Muhammad, who is the greatest educator, the everlasting source of knowledge and guidance to the whole of the humanity.

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Our selfishness has sacrificed millions and millions of bacteria. May a time will come when we can save their sole. I feel sorry for their harvest.

Muneeba Waseem Ghauri.

ABBREVIATIONS

EDTA	Ethylene diamine tetra acetic acid
NaOH	Sodium Hydroxide
NaCl	Sodium Chloride
μL	Micro liter
UV	Ultra violet
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
pH	Paviour of hydrogen
OD ₆₀₀	Optical density at 600 nm
Nm	Nano meter
LB	Lauria Bertani
Mm	Milli molar
Kb	Kilo base

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INTRODUCTION

1 INTRODUCTION

The roles of bacterial extremophiles, for some time, have been underestimated. This is somewhat for the, for example, there is the occurrence of degradation of petroleum deposits deep in the planet, while in deposits of coal, groups of Archaea and Bacteria cooperate to produce methane. Bacterial extremophiles have been exploited by the Biotechnologists as whole organisms to speed up the processes under extreme situations or as a source of enzymes. *Thermus aquaticus* being the thermostable DNA polymerase is the most renowned example enabling it to use it in the polymerase chain reaction (PCR), involving continual reactant cycles between $\sim 50^{\circ}\text{C}$ and 95°C . It has now turned out to be one of the basic devices for the research on the extremophilic microbes. Even though thermophiles produce many extremozymes, various valuable enzymes have additionally originated from acidophiles (amylases for acidic hydrolysis of starch), psychrophiles (lipases and proteases as detergent additives active at low temperature), halophiles (their enzymatic activity in in non-aqueous solvents) and alkaliphiles (proteases for detergent applications). alkaliphiles and Acidophiles naturally secrete enzymes which tend to be extremophilic and also maintain their cytoplasm at neutral pH. In comparison, just by two simple mechanisms halophiles adjust to the osmotic pressure either by reducing the osmotic stress by accumulating the increased amount of neutral organic species of low molecular weight or sustaining a higher concentration of salt in the cytoplasm. In the earlier strategy, according to the environment the proteins in the cytoplasm are adjusted, which causes precipitation of proteins by gathering negatively charged amino acids at the surface, as well as supporting their activity in non-aqueous solvents and stability.