BIOPROSPECTING FOR CULTURABLE PSYCHROTROPHIC BACTERIA WITH EXTRACELLULAR HYDROLYTIC ENZYMES ACTIVITY FROM ARCTIC LAKE SEDIMENTS

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I dedicate this work to my deceased father and mother: Alhaji Idris Yusuf and Fatima Idris. May Allah forgive their shortcomings and make Jannatul-Firdaus to be their final abode.

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ABSTRACT

The ability of cold-adapted microorganisms to produce cold-active enzymes with potential biotechnological applications has recently attracted the attention of scientific community in terms of bioprospecting. This research aimed at assessing the presence and diversity of culturable psychrotrophic bacteria with hydrolytic enzymes activity and their identification using molecular approach. A total of six (6) different Arctic lake sediments were analysed. Mean viable bacterial count ranged from $2.88X10^3$ to $5.07X10^5$ cfu/g. A total of thirty seven (37) bacterial strains were successfully isolated at 20°C and screened for Amylase, Protease and Lipase activity. Molecular characterization using 16S rRNA gene sequence homology revealed that the isolated bacteria belong to seven genera comprising *Pseudomonas, Bacillus, Dermacoccus, Arthrobacter, Janthinobacterium, Paenibacillus* and *Chryseobacterium.* Eighteen (18) isolates; representing 49% were found to be positive to at least one of the three enzymes activity tested. Two different strains of *Pseudomonas sp*. (isolates 16D4 and 17D4) were found to be the most potent protease and lipase producing isolates respectively. XIA12 identified as *Bacillus cereus* was found to be the most potent amylase producing bacteria. Study of the growth temperature of the isolated bacteria revealed that most of isolates could grow at temperature above 20°C signifying that the isolates are true psychrotrophs. The findings of this work have confirmed that Arctic environment can serve as an ideal area for biotechnological exploration.

ABSTRAK

Baru-baru ini, keupayaan mikroorganisma sejuk untuk menghasilkan enzim sejuk-aktif yang mempunyai potensi dalam aplikasi bioteknologi telah menarik perhatian para saintifik dari segi bioprospek. Kajian ini bertujuan untuk menilai kehadiran dan kepelbagaian bakteria psychrotrophic yang boleh diternak dengan aktiviti enzim hidrolitik dan pengenalan bakteria menggunakan pendekatan molekul. Sebanyak enam (6) sedimen tasik Artik yang berbeza telah dianalisis. Purata kiraan bakteria yang boleh dicapai adalah di antara $2.88X10^3$ hingga $5.07X10^5$ cfu/g. Sebanyak tiga puluh tujuh (37) jenis bakteria telah berjaya diasingkan pada 20°C dan ditapis untuk aktiviti Amilase, Protease dan Lipase. Pencirian molekul menggunakan 16S rRNA homologi urutan gen mendedahkan bahawa bakteria yang telah diasingkan tergolong dalam tujuh genera yang terdiri daripada *Pseudomonas, Bacillus, Dermacoccus, Arthrobacter, Janthinobacterium, Paenibacillus dan Chryseobacterium*. Lapan belas (18) bakteria yang telah diasingkan; sebanyak 49% didapati positif untuk sekurang-kurangnya salah satu daripada tiga ujian aktiviti enzim. Dua jenis bakteria yang berbeza daripada golongan *Pseudomonas sp.* (pengasingan 16D4 dan 17D4) masing-masing didapati paling berkesan dalam menghasilkan protease dan lipase. XIA12 telah dikenal pasti sebagai *Bacillus cereus* sebagai bakteria yang paling berkesan dalam menghasilkan amilase. Kajian suhu pertumbuhan bakteria yang telah diasingkan mendedahkan bahawa sebahagian besar daripadanya boleh hidup pada suhu melebihi 20°C menandakan bahawa ianya adalah psychrotrophs yang benar. Hasil kerja ini telah mengesahkan bahawa persekitaran Artik boleh berfungsi sebagai kawasan yang sesuai untuk eksplorasi bioteknologi.

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LIST OF ABBREVATIONS AND SYMBOLS

ABM	-	Antarctic Bacterial Medium
ABI	-	Application Binary Interface
AFPs	-	Antifreeze Proteins
BLAST	-	Basic Local Alignment Tool
CAPS	-	Cold Acclimation Proteins
cfu/g	-	Colony Forming Unit
CaCl2	-	Calcium Chloride
°C	-	Degree Celcius
DNA	-	Deoxyribonucleic Acids
EDTA	-	Ethylene Diamine Tetraacetic Acid
SMA	-	Skim Milk Agar
g	-	Gram
gb	-	GenBank
g/ml	-	Gram Per Millilitre
h	-	Hour
Hc	-	Coefficient of hydrolysis
H_2O	-	Water
bp	-	Base pairs
kbp		Kilo base pairs
L	-	Litre
Min	-	Minutes
mg/ml	-	Miligram per milliliter
$MgSO_4$	-	Magnesium Sulphate
ml	-	Mililitres

mM	-	Milimolar
mm	-	Milimetre
M	-	Molar
ng	-	Nanogram
NCBI		National Center for Biotechnology Information
NA	-	Nutrient Agar
NB	-	Nutrient Broth
NaCl	-	Sodium Chloride
NaOH	-	Sodium Hydroxide
ng/µL		Nanogram Per Microliter.
%	-	Percent
PCR	-	Polymerase Chain Reaction
PUFAs		Polyunsaturated Fatty Acids
rpm	-	Revolutions Per Minute
sec	-	Seconds
TAE	-	Tris acetate EDTA
UV	-	Ultraviolet
µL	-	Microlitre
v/v	-	Volume per volume
w/v	-	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The term bioprospecting has been described as a systematic search for novel microorganisms, bioactive compounds, proteins, genes and other products with promising commercial applications (Pascale *et al.*, 2012). The potential for isolating new microbial species for biotechnological applications has initiated a strong interest in the microbiology of cold environment such as Arctic and Antarctic polar regions (Männistö and Häggblom, 2006). Several new bacteria have been isolated, identified and characterized within the past few years, from permanently cold environment. In recent times, potential applications of cold adapted microorganisms and their metabolic products including cold-active enzymes and novel bioactive compounds have been documented (Deming, 2002).

Psychrotrophic bacteria, which are distinct from psychrophilic bacteria due to their ability to grow both at 4°C and at 20°C, have been known to be widely distributed in natural environment and they adapt more to a wider growth temperature range than psychrophiles (Radjasa *et al.*, 2001). A large proportion of the earth's surface is occupied by cold environment such as the Arctic, Antarctic, alpine regions, and abysses. Due to the extremely harsh climatic conditions, these cold environments have been considered for a long time to be extreme and therefore

devoid of life or serving only as repositories for wind-transported microbes trapped in the frozen water (Cowan and Tow, 2004). Although still limited, the growing number of recent studies on the microbial diversity and ecology of cold habitat have changed this view, they have revealed that, even under such hostile conditions associated with polar regions, such areas harbour abundant, live and diverse microbes; particularly psychrotrophs and psychrophiles that may be detected and recovered by cultivation (Suzuki *et al.*, 2001). Recently, increasing attention has been paid to the application of psychrophiles, psychrotrophs, and their cold active enzymes in biotechnology. For example, cold active hydrolases such as lipases, proteinases, amylases and cellulases from these microorganisms have been used as additives in laundry detergents (Suzuki *et al.*, 2001). Potential applications of cold-adapted microorganisms may also include their use as agents for bioremediation at low temperature as well as mitigation greenhouse gas effect.

Furthermore, low temperature environment are abundant on Earth and have been well inhabited by cold-adapted microorganisms most of which can be utilise as cell factories for the production of valuable chemical compounds as well as for clean-up of polluted cold environments (Margesin and Feller, 2010). Likewise, the biomolecules produce by these organisms, particularly proteins and enzymes have already found useful applications in various fields such as medical research, molecular biology, industrial food or feed technologies, detergents and cosmetics (Margesin and Feller, 2010).

In the recent years, the study of cold environments such as arctic and their microorganisms especially bacteria has begun to receive greater attention through which variety of native microbial life forms have been discovered and characterized. (Gesheva and Negoita, 2012). Although arctic environment is sometimes being described as inhospitable, recent applications of molecular methods have revealed a very wide diversity of microbial taxa in the samples taken from such environments; many of which are yet to be cultured and taxonomically unique. Therefore, these environments could be considered to be a suitable environment for the isolation of bacteria capable of producing cold-active enzymes; some of which have been found to be useful in industry (Martínez-Rosales and Castro-Sowinski, 2011).

There have been numerous studies to determine microbial diversity in polar region through culture-dependent methods. For example, bacteria have been recovered from the arctic tundra soils in Siberia, Svalbard, and Canada; majority of which belonged to the phylum *Actinobacteria, Bacteroidetes, Firmicutes*, and *Proteobacteria* (Kim *et al.*, 2013). It is important to acknowledge that culture-based methods underestimate the true diversity of bacteria associated with environmental samples. However, approaches to investigating microbial ecosystems on the basis of conventional culture methods are important since, via this approach, the ecological role of the cultivated and characterized microbes can be estimated. Furthermore, culturing is necessary to biochemically classify and analyse some physiological features of the organisms for biotechnological exploitation (Galkiewicz *et al.*, 2011).

1.2 Problem Statement

Bioprospecting of cold-adapted microbial resources has been a real challenge and opportunity for contemporary biotechnology. Cold-adapted bioactive compounds recovered from psychrophilic and psychrotrophic microorganisms continue to provide advantages in different areas, such as energy savings; activity at low temperatures; the possibility of challenging reactions with a sufficiently high rate; efficient production with lower cost of processing; thermal protection as well as improving quality of products. The market for bioactive products and industrial enzymes at the moment is growing. Cold-active hydrolytic enzymes such as lipases, proteases amylases etc. have numerous potential applications especially in industries.

Despite an increasing number of microbial diversity assessments of Polar Regions that began to accumulate in recent years, only relatively little is known about the cold-adapted hydrolytic enzymes producing bacteria. Also, early studies of bacteria in Arctic environmental samples have focused much on abundance and diversity and to a certain extent; the influence of climatic conditions on these microbes with relatively few information about the potentials of the organisms

recovered from such environment; in terms of production of bioactive compounds for biotechnological applications. Thus, cultivating and characterizing isolates are believed to be particularly important for providing insight into diversity and bioactivity of these organisms especially; because culture-based methods make the characteristics of microorganisms clear, and also provides several interesting information such as the low temperature adaptability of organisms, elaboration of extracellular enzymes, and many other physiological activities. It is therefore based on this background that this research is designed with the aim of investigating the presence and diversity of psychrotrophic bacteria with extracellular hydrolytic enzymes activity for possible environmental and industrial applications.

1.3 Objectives of the Research

The specific objectives of this research are:

i. To enumerate and isolate culturable psychrotrophic bacteria associated with Arctic lake sediments using standard culture-based methods.
ii. To characterize and identify the isolates using 16S rRNA gene sequence homology.
iii. To screen the isolated bacteria for selected extracellular hydrolytic enzymes activity.

1.4 Scope of the Research

The scope of the research includes: investigating the presence and diversity of culturable psychrotrophic bacteria through enumeration and isolation of bacteria associated with Arctic sediment using standard microbiological methods. Pure cultures of each isolate were identified and characterized using molecular approach

(16S rRNA gene sequence homology), the isolated bacteria were screened for some selected hydrolytic enzymes activity; namely amylase, lipase and protease.

1.5 Significance and Original Contributions of the Research

Industrial processes are carried out under specific conditions which in most cases cannot always be accustomed to an optimal values required for the activity of the enzymes available (Cojoc *et al.*, 2009). The increased use of microbial enzymes especially bacterial extracellular hydrolases in the industry has focused attention on the source identification and recovery of novel enzymes with new desirable properties such as low temperature performance. Such effort is very vital in terms of saving energy as some industrial processes could chiefly be accomplished at near or room temperature (Martínez-Rosales and Castro-Sowinski, 2011). Cold-adapted enzymes have generally been observed to possess high specific activity or catalytic efficiency at low and moderate temperatures relative to enzymes derived from mesophilic or thermophilic microorganisms (Margesin *et al.*, 2008).

The capacity of microbes to synthesize enzymes capable of catalyzing reactions at temperatures near freezing point of water is no small feat. Given the commercial success of these special enzymes, effort is now being directed toward isolating microbes particularly bacteria capable of producing such enzymes in commercial quantity. This research has the potential to make a significant contribution to the existing knowledge of bacterial diversity of arctic ecosystems as well as the potentials of the indigenous microbes in search for a novel biocatalyst with improved properties.

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