SCREENING AND CHARACTERIZATION OF CELLULOLYTIC BACTERIA ISOLATED FROM ARCTIC SOIL AND SEDIMENT SAMPLES

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This research work is specially dedicated to my beloved parents, and the entire Muslim.

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ABSTRACT

The study on microorganisms living in extreme cold environment such as the arctic and antarctic regions is important for the biotechnological exploration and biodiversity. Such organisms however, could have enzymes capable of degrading complex molecules at low temperature. In this work, culturable bacteria from arctic soil and sediment samples have been isolated under low temperature (20 °C), using Antarctic Bacterium medium (ABM). A total of 40 different bacteria were isolated and screen for their ability to produce cellulase using carboxymethyl cellulose (CMC) as substrates. Cellulase producing bacteria were selected based on their hydrolytic coefficient. Molecular characterizations of the potent cellulase producing isolates were carried out using 16S rRNA gene sequence method. The effects of temperature, pH and salinity were tested on four bacterial isolates that showed high cellulase activity. However only two of the isolates (SL9 and SL 19) were able to retains their cellulolytic activity at 5%, 10%, and 15% salt concentrations. Based on the 16S rRNA gene sequence and the phylogenetic analysis, the four potential cellulolytic bacterial isolates were identified as *Massilia* sp. *and Pseudomonas* sp. The findings of this work suggested that arctic bacterial isolates such as *Massilia* and *Peudomonas* species could serve as promising agents for the production of bacterial cellulase with various biotechnological applications; such as food supplement, animal feed supplement as well as bioremediation of pharmaceutical wastewater.

ABSTRAK

Kajian terhadap mikroorganisma yang hidup dalam persekitaran sejuk melampau seperti kawasan artik dan antartika adalah penting untuk penerokaan bioteknologi dan biodiversiti.Walaubagaimanapun terdapat mikroorganisma yang mempunyai enzim yang mampu mengurangkan molekul kompleks pada suhu rendah. Dalam kajian ini, bakteria kultur dari tanah artik dan sedimen sampel telah diasingkan di bawah suhu rendah (20 °C), dengan menggunakan bakteria Antartika sederhana (ABM). Sebanyak 40 bakteria yang berbeza telah diasingkan dan disaring untuk menghasilkan selulase menggunakan kuvboksimetil selulosa (CMC) sebagai substrat. Bakteria menghasilkan selulase dipilih berdasarkan pemalar hidrolitik mereka. Pemilihan berdasarkan kriteria molekul yang kuat menghasilkan selulase telah dijalankan menggunakan kaedah urutan gen 16S rRNA. Dari kesan suhu, pH dan kemasinan telah diuji ke atas empat isolat bakteria yang menunjukkan aktiviti selulase tinggi. Walau bagaimanapun hanya dua daripada bakteria (SL9 dan SL 19) dapat mengekalkan aktiviti cellulolytic mereka pada 5%, 10%, dan 15% kepekatan garam. Berdasarkan urutan gen 16S rRNA dan analisis filogenetik, empat bakteria cellulolytic berpotensi telah dikenal pasti sebagai *Massilia* sp. dan *Pseudomonas* sp . Cadangan daripada hasil kerja ini ialah bahawa isolat bakteria artik seperti *Massilia* dan *Peudomonas* spesis boleh dijadikan agen untuk pengeluaran enzim bakteria dengan pelbagai aplikasi bioteknologi; seperti makanan tambahan, makanan tambahan ternakan serta bioremediasi air sisa farmaseutikal.

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CMC	-	Carboxymethyl Cellulose
ABM	-	Antarctic Bacterium Medium
DNA	-	Deoxyribonucleic Acid
RNA	-	Ribonucleic Acid
et al.	-	And others
cm	-	centimeter
g	-	grams
ml	-	Milliliter
nm	-	Nanometer
L	-	Liter
pH	-	Hydrogen ion concentration
rpm	-	Rotation per minute
v/v	-	Volume by volume
w/v	-	Weight by volume
%	-	percent
$^{\circ}C$	-	Degree Celsius
PCR	-	Polymerase Chain Reaction
rRNA	-	Ribosomal ribonucleic acid
BLAST	-	Basic Local Alignment search Tool
NCBI	-	National Centre for Biotechnology information
sp.	-	species
β	-	Beta
H_c	-	Hydrolytic coefficient

µl	-	Microlitre
EtBr	-	Ethidium Bromide
UV	-	Ultra violet

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Cold environment are the most abundant environment on the planet surface which has been successfully colonizes by a vast number of microorganisms. Bacteria, Fungi, Unicellular Algae, and Yeast are the most diverse group due to their closed internal temperature, when it is not identical to their surrounding environment. In spite of the durable negative consequence of low temperatures on biological reactions, these organisms sort, grow and change at rates comparable to those attained by closely related class existing in temperate surroundings. They have consequently developed numerous adaptations in the form of wonderfully adjusted organizational changes at their cellular level, for example, their membranes, constitutive proteins and enzymes, enabling them to compensate for the deleterious effects of low temperature (Gerday *et al*., 2000).

The extreme cold environment have been found to contain several habitats such as sea water , ice, and permafrost, which can serve as the region potentially used for isolation of bacteria with biotechnological abilities (Reddy *et al*., 2009). The cold regions have become a model system for global warming and are regarded

as key European sites for biodiversity monitoring (Reddy *et al*., 2009). The extreme cold region such as arctic and antarctica have been found to constitute more than 14% of the biosphere and are the most coldest and most arid environment on earth (Møller *et al.*, 2011). The structure and function of arctic soil microbial communities with the various numbers of glaciers contained by arctic, little is known about the diversity of bacteria in the glaciers (Reddy *et al*., 2009).

Various range of microbes have been revealed to be found in cold environment and comprises of representatives of the bacteria, eukaryotes and Achaea (Cavicchioli, *et al*., 2002). Microorganisms found to adapt low - temperature for their optimal growth condition under natural and artificial condition are divided into psychrophiles which are capable of growing at temperature above 20°C and psychrotrophs those that can grow optimally at temperature between 15°C and 30°C (Kato, *et al.*, 2001) . The psychrotrophic microorganisms that have the potential in natural bioremediation of certain organic compounds such as hydrocarbons in the soil , water, and marine environment are cold adapted and metabolically poorly understood , despite their importance in bioremediation (Kato *et al*., 2001)

The survival of microorganisms to harsh environmental conditions has made them very promising source for bio prospecting. These environmental conditions are extensively described as drivers for several processes in existing organisms; this contributes to microbial selection for differential enzymatic activity. Stressing factors have been divided into physical (pressure, temperature, and radiation), and geochemical (pH and salinity) which are the contributing factors for microbial diversity (Van Den Burg, 2003). Soil microorganisms, has been found to play an important role in bioconversion and degradation of organic matter. Soil bacteria has the ability to decomposed cellulose- based materials (Soares *et al*., 2012). The resistance to extreme temperatures, pH and pressure by the microorganisms and synthesis of particular enzymes are necessary for their adaptive response (Clarke, 2003). The microbial presence in such condition are of enormous importance for the exploration of biodiversity in biotechnology (Soares *et al*., 2012)

Cellulases as the term refer to the group of enzymes that can catalyze the hydrolysis of cellulose into sugars. Therefore cellulolytic microorganisms plays and important role in the biosphere by recycling cellulose, which is the most abundant carbohydrate produced by plant (Kasana *et al*., 2008). Microorganisms with cellulolytic enzymes have many potential biotechnological as well as industrial applications; it is therefore required in a large quantities due to their industrial applications, such as; detergent, animal feed, textile biofuel, paper and pulp, waste management and pharmaceuticals (Kasana *et al.*, 2008).

The study of cultivable cold adaptive cellulases producing bacteria are of metabolically important owing to their potential in biodegradation, these group of bacteria have been found to contain various enzymes with bioremediation and industrial applications (Rashid *et al*., 1999). The enzymes with thermo stable activity are of great importance for industries (Rashid *et al*., 1999). However enzymes with little stability at elevated temperature are favourable for some purposes especially in certain reaction which can only be activated at low temperature. Sources such as arctic and antarctica are expected to contain microorganisms that can produce cold adapted enzymes (Rashid *et al*., 1999). The properties of cold-active enzymes may be responsible for various industrial applications, though, specific properties may be enhanced through enzymes engineering (Cavicchioli *et al*., 2002).

The 16S rDNA sequence is the most widely used to study bacterial phylogenetic relationship due to its presence in almost all bacteria existing as a multi genic family or their operons (Janda and Abbott, 2007). The gene sequencing of the 16S rDNA in the laboratory has been considered to be reliable method for identification of unidentified bacteria biochemically by providing reference from the strain of related species (Janda and Abbott, 2007). The 16S rDNA sequence has an intensifying role in the proof of identity of bacteria, but it is not fool proof and relevant in each and every situation (Janda and Abbott, 2007). It is imprecise, however, whether the outcomes from laboratory investigation with these categories of bacteria can be generalized to the extreme cold environment, since the bacteria originated from the arctic and other cold region remain only remotely associated to

the *gamma-proteobacteria* (*Escherichia .coli* and *vibrio*) and other cultured bacteria inspected in the laboratory experimentations conducted to date (Kirchman *et al.*, 2005).

1.2 Problem statement

Microbial life have proved not limited to specific environments, as microorganisms can be found in the most diverse area with extreme conditions, such as temperature, salinity , pH, and pressure. Extremophiles produced functional biocatalyst under extreme condition; the unique properties of this biocatalyst have impacts on industrial processes (Van Den Burg, 2003). The presence of microorganisms living in extreme cold environment such as arctic and antarctic regions is important for the biotechnological exploration as well as biodiversity. From a biotechnological perspective, it might be useful to study organisms found in harsh environments. Such organisms could have enzymes that can reduce complex molecules, like cellulose, which is the most ample renewable energy source on Earth (Soares *et al*., 2012). However, the complete degradation of cellulose requires the action of various enzymes, cooperatively called cellulases, due to its complex assembly.

The screening of cellulolytic bacterial community isolated from cold environment is of immense importance owing to their biotechnological potentials, which may lead to the enzymes discovery and the improvement of their biotechnological applications. This group of microorganisms are anticipated to produce several numbers of cold adapted enzymes such as cellulases and other hydrolases. Though, there have been limited number of reports on the screening and characterization of bacteria with cellulolytic activity from extreme cold environment, which may be found to have the potential to play a significant role in bioremediation as well as industrial applications. Therefore, it is in the light of the above; that this

research is designed with the aim of isolating psychotropic bacteria with cellulolytic activity from the arctic soil and sediment samples. There is a need to further the study and characterize bacteria from such environments. This research will further explore in the search for cellulolytic enzymes to be used in bioenergy industries. At present-day, only a slight portion of the microorganisms on Earth have been exploited.

1.3 Objectives of the study

This research was aimed at isolating psychotropic bacteria with cellulolytic activity from the arctic soil and sediment samples and test the effects of growth parameters for their activity. The main objectives of the study are;

1. To isolate and screen for bacteria with cellulolytic activity from arctic soil and sediment samples

2. To test the effect of pH, temperature and salinity on the bacterial cellulolytic activity

3. To identify bacteria with high cellulolytic activity using the 16S rRNA gene sequencing method

1.4 Scope of the research

This research was focused on the isolation, screening, characterization and identification of cellulolytic bacteria associated with extreme cold environment (arctic region) , the bacteria with the high cellulolytic activity among test isolates were identified further using 16S rDNA characterizations methods, The bacteria identified were further subjected to different temperature condition, pH and salinity to observe the effects of the physical parameters on the cellulolytic activity of the isolates.

1.5 Significance of the research

The extreme cold environment has been identified as major source of new natural products and also serves as the source for bio-prospecting of bacteria with cellulolytic activity. It harbours many bacteria that survive under different low temperature. This study when completed will give an insight to the understanding of the role of bacteria isolated from cold environment as well as their ability to produce cellulolytic enzymes under low temperature. This study will serve as starting point to further the research on enzymes produced by bacteria that can be used for biotransformation at low temperature and further promote the industrial applications of cellulase in the production of animal feeds, textiles, detergents and food processing industries as well as bioenergy industries.

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