Bima Journal of Science and Technology, Vol. 5(2) Dec, 2021 ISSN: 2536-6041

MOLECULAR IDENTIFICATION OF PSYCHROTROPIC *BACTERIA* FROM ANTARCTIC SOIL

[1*]ISMAIL HARUNA, [1]NAZEEF IDRIS USMAN AND [1]ALIYU ADAMU

[1]Department of Microbiology, Bauchi State University Gadau, PMB 65 Gadau, Nigeria.

*Corresponding Author: ismailharuna@basug.edu.ng, ismailharuna68@yahoo.com

ABSTRACT

Antarctic environment is one of the most fragile ecological systems on the earth planet. Literature indicated that a large number of reviews on diversity of microbes in cold environments often begin with a lamentation on the inadequate information about organisms that leave in cold areas or their ways of adaptation to such environments. Our objective was to identify psychrotrophic bacteria from Top cliff soil collected from Antarctica. Bacterium was isolated by incubating the sample in Antarctic Bacterial Medium in the laboratory at 20^{o}C for 48 hours. Promega, USA DNA purification kit was used to extract the bacterial DNA. Universal eubacterial primers 27F and 1492R were used to amplify the 16S rRNA gene of the bacteria. The Amplified genes were sequenced and analysed using the basic local alignment search tool (BLAST) to identify the bacterium with highest similarity and phylogenetic tree was contrusted using clustalX. Results of the gel electrophoresis of the PCR product produced DNA fragment bands of approximately 1500bp. The isolate share 97% similarity with the top match in the GENBANK. *Bacillus* species were found having highest percentage similarity with the isolated bacterium from Antarctic. The 16S rRNA partial gene sequence of the psychptrophic bacterium isolated in this study was successfully deposited in GenBank nucleotide database by direct submission and receive the KX000297 accession number.

Keywords: Psychtrophs; Antarctic Bacterial Medium; DNA Extraction; Gene Amplification; Phylogeny;

INTRODUCTION

Relatively, Antarctica is considered as unknown and is of significant interest for bioprospecting. The community structure and diversity of Antarctic bacteria have been studied by various methods(Ganzert *et al.*, 2011; Yergeau *et al.*, 2009);. allowing the study of different characteristics of bacterial communities. Molecular identification of bacteria from this environment reveal that the majority of the isolated microorganisms are new species (Russell, 2006).

The definitions of the term psychrotrophic as proposed by Morita have been considered to be the most precise and widely accepted (Radjasa *et al.*, 2001). Psychrotrophic bacteria have the ability to grow both at 20°C and sometimes they show an upper growth-temperature of 40°C (Moyer and Morita, 2007) 40°C. This feature makes them to be distributed widely in natural environments and adapt to a wide range temperature for growth.

The soils of Antarctica are severe environments inhabited by well adapted microorganisms, the knowledge of which is scarce. The study on their abundance, diversity and physiology will provide the investigators with new data on the mechanisms of their adaptation and gives new opportunities to isolate microorganisms with unique properties for practical use(Sjöling and Cowan, 2003).

MATERIALS AND METHODS

Sample Collection

Soil sample was obtained from University Teknologi Malaysia environmental biotechnology research group of Faculty of Bioscience and Medical Engineering. The soil was previously collected from arctic region around Svalbard Ireland (Latitude 77°N, Longitude 15°E). The sample was successfully taken to the laboratory under sterile conditions.

Isolation of Psychrotriphic Bacteria

About 5g soil was suspended in 150 ml of distilled water. Using streak plate method, the solution was transferred onto the Antarctic Bacterial Medium plates (Haruna, 2019; Kharsany *et al.*). The Plates were incubated at 20°C for two days. Formation of colonies was observed.

Genomic DNA Extraction from Bacterium Isolate

Promega, USA DNA purification kit was use in accordance with the instructions of manufacturer to extract the genomic DNA of the isolated bacteria. An aliquot (1ml) taken from broth culture of the test isolate and was transferred into micro centrifuge tube with 1.5ml capacity and centrifuged for 2 minutes at 13000 rpm; after which the supernatant was removed carefully. The cells were resuspended in 50mM EDTA (480uL), followed by addition of 120uL lysozyme and incubated for 30 min at 37^0 C and another centrifugation at 13000 rpm for 2 minutes, the supernatant is removed. The cell was suspended again by adding 600uL Nuclei lysis solution with gentle pippeting. The resuspended cells were incubated at this point at 80°C for 5 minutes. Cooling at 25°C is followed by addition of 3uL RNase solution to the cell lysate, for proper mixing, followed by inverting the tube 2-5 times. It was cooled to room tempreture after incubation for 15-60 min at at 37°C. Addition of 200uL protein precipitation solution to the lysate and vigourously mixed for half a minute, and keep on ice for 5 minutes. It was centrifuged for 3 minutes at 13000 rpm after which supernatant containing isopropanol (600uL). The tube was inverted two times for mixing before centrifugation at 16000 rpm for 2 min. The isopropanol was discarded. 600uL of 70% ethanol was added to wash the pellet and the tube was inverted several times and then centrifuged at 16000 rpm for 2 minutes. The ethanol was discarded and the DNA pellet allowed drying. Lastly, the extracted DNA was rehydrated using DNA rehydration solution (100u) and incubated overnight at 4°C.

Purity Assessment and Quantification of the Extracted DNA

The genomic extracted DNA purity and quantity was assessed using Nano Drop ND-1000 spectrophotometer (thermo scientific, USA). The blank used was DNA rehydration solution (PROMEGA). 1uL of DNA sample was spectrophotometrically quantified following the operating machine procedure as specified by the manufacturer.

Amplification of 16S rRNA Gene Amplification Using Polymerase Chain Reaction

The genomic DNA extracted from the bacterial isolate was used as a template for specific amplification of 16S rRNA gene fragment using polymerase chain reaction. Universal eubacterial primers 27F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' CGGTTACCTTGTTACGACTT 3') were used for amplification. PCR was performed in a 50uL volume using template DNA, 2uL each of forward and reverse primer; 19uL nuclease free water, 25uL master mixer containing dNTP, Taq DNA polymerase (PROMEGA) and $MgCl_2$. The reactions were

accomplished in the thermocycler (Eppendorf, USA) with cycling parameters of 95°C for 30 minutes initial denaturation, then 30 cycles of 95°C for 1 minute, followed by 55°C for 60 seconds, 72°C for 2 minutes, and the final extension was performed at 72°C for 10 minutes(Boesenberg-Smith *et al.*, 2012).

Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed to evaluate the success of 16S rRNA gene amplification. Agarose (0.4g) was dissolved in 50ml TAE buffer; the solution was then heated in microwave for 1min to achieve absolute homogeneity. 2uL ethidium bromide was added to the prepared agarose solution and poured into the gel tray carrying the gel comb to create the loading wells, the gel was allowed to set for about 45 minutes after which the comb was carefully removed and the gel was transferred into the electrophoretic tank containing TAE buffer. 5uL DNA ladder solution was loaded into the first well of the gel. For sample loading, 4uL PCR product was in each case mixed with 2uL loading dye on a parafilm and then transferred into the wells. The gel electrophoresis was run at 80V for 50 minutes. To check for the presence of 16s rRNA gene fragment which is about 1500bp. lastly the gel was visualized in Gel view system under UV irradiation.

16S rRNA gene Sequencing and phylogenetic analysis

The PCR product was sent to FIRST BASE Laboratories Sdn Bhd (Selengor-Malaysia) for purification and sequencing. The gene sequence obtained was then visualized and assembled using ChromasPro version 1.6 (Technelysium Pty Ltd, South Brisbane, Queensland, Australia) and Bioedit Sequences Alignment editor Version 7.2.5 (Hall *et al.*, 2011). The assembled sequence was used to query the NCBI BLAST database at NCBI (www.ncbi.nlm.nih.gov/blast) in order to determine the taxonomic group of the isolate by way of sequence homology and comparison with Gen Bank top hits of the BLAST search result(Madden, 2003). Finally, the phylogeny.fr was used to drive phylogenetic relationship for identification of the isolated bacteria.

RESULTS AND DISCUSSION

Isolation of Psychrotropic Bacteria

A psychotropic bacterium was isolated from the top cliff Antarctic soil on ABM. The bacteria grow within 24 hours at 30°C and 48 hours when incubated at 20°C. The bacterial colony was observed as smooth surface and formed milky colonies. This is due to low metabolic rate associated with the growth at low incubation tempreture (Feller and Gerday, 2003; Nichols *et al.*, 1999). The pigmentation (milky appearance) play a major role in the survival of microorganisms in cold environment (D'Amico *et al.*, 2003).

Identification of Isolated Bacteria Using 16S rRNA Gene Sequence

Identification of the isolated bacteria was achieved after 16S rRNA gene amplification and sequencing. Genomic DNA of the isolate was successfully extracted. The concentration of the genomic DNA extracted in ng/ul was mostly between 148 and 250; and the DNA purity (A_{260}/A_{280}) recorded was between 1.84 and 1.96. These values could be considered appropriate for an optimum PCR reaction; because they fall within limit of 1.8 to 20 (Boesenberg-Smith et al., 2012). Assessing the purity of extracted DNA is very critical because it affects the overall success of the amplification (He, 2011).

The PCR amplifications of the 16S rRNA gene of the isolated bacterium was successfully done. Success of the amplification was evaluated using agarose gel electrophoresis and visualization of the Gel under UV light. Results of the gel

electrophoresis of the amplified PCR product produced light DNA fragment bands of approximately 1500bp when compared with the standard ladder. Figure 4.8 shows the light DNA bands of the isolate with control showing no light band. Absence of light DNA band in the case of control path indicates that the band obtained for the test sample was actually as a result of successful amplification of the target genes (16S rRNA gene) from the template DNA (genomic DNA) (Nogales et al., 2001).

16S rRNA Gene sequences Analysis

The gene sequence for the isolate was received from First Base Laboratory Sdn. Bhd. in form of ABI file. The chromatogram was viewed in using Chromaspro version 1.6 (Technelysin pty Ltd, South Brisbane, Queensland, Australia). 16S rRNA partial gene sequence of isolate was obtained by multiple sequence alignment, editing and assembly of the forward and reverse sequences using Bioedit sequence alignment Editor version 7.2.5. BLASTn search using the assembled sequences as query led to the identification of the isolated bacteria (Madden, 2013). The isolated bacterium was identified using its gene

The sequence was blast in the NCBI nucleotide data base. The isolate shares 97% similarity with the top match in the GENBANK which is significant enough (Madden, 2013). From percentage of the similarity the bacteria were found to be bacillus. This is in accordance with who isolated and 32 bacterial strains from Antarctic including bacillus. Information from the GENBANK show two bacteria (*Bacillus spp* clone C6A08; *Bacillus cereus strain ASBCFS39)* out of the top ten match from the blast were isolated from estuary which is also a cold environment. Four were isolated from soil (*Bacillus spp. E54; Bacillus anthracis strain NX4; Bacillus sp D37; Bacillus* cereus strain *DYJL1).* We could not find information about where the soil was gotten from but it still shows similarity with our isolate since is also gotten from soil. The remaining three isolate *Bacillus* cereus *strain SE1; Bacillus anthracis strain 711; and Bacillus sp. Hb62* were gotten from solid waste, fresh leaf and honey respectively (http://www.ncbi.nih.gov). The phylogenetic tree was constructed using 16S rRNA gene sequences of the related bacterial strains obtained from the GenBank database (http://www.ncbi.nih.gov) using phylogeny.fr. Phylogenetic analysis based on 16S ribosomal RNA indicates a close relationship with *Bacillus sp hb89*. Figure 1 below shows the phylogenetic trees constructed base on Neighbour-joining method. Our bacteria in the middle of the tree with the name isolated indicated by blue line. However, the closest bacteria with our isolate in the phylogenetic tree (*Bacillus sp hb89*) were not the closest when we blast the sequence The result from our blast shows the closest bacteria to our isolate to be *Bacillus Sp hb62* (Kim *et al.*, 2011). We search and found that both of the isolate are from the same source.

Nucleotide Sequence Accession Number

16S rRNA partial gene sequence of the psychptrophic bacteria was successfully deposited in GenBank nucleotide database by direct submission with KX000297 accession number. The bacteria has 596 bases and it DNA is linear as describe in the data base with db_xref="taxon:1822212". The bacterial data base id was assigned as SEQBCIH. The isolate was named as *Bacillus sp. Ih1 with accession number* KX000297. Strain was isolated from soil in Malaysia. Table 1 below shows the details of the deposited sequence.

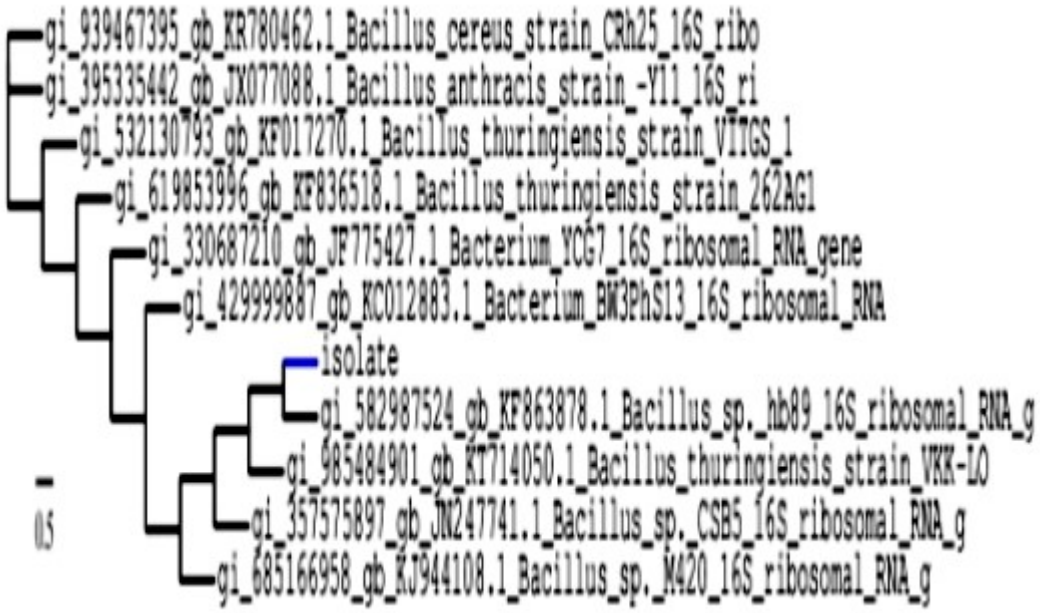

Figure1: Phylogeny generated using Neighbour-Joining method

Table 1: The Details of the Deposited Sequence

Isolate ID	Strain Name	Gene Bank Accession Number
SEQBCIH	Bacillus sp. ih1	KX000297

Our main goal was to isolate psychrotrphic bacteria from Antarctica. Top cliff soil sample collected by utm research team from Antarctica was cultured on ABM. 16S rRNA identification method, the isolate was identified as bacillus sp. The strain bacillus is believed to be one of the dominant and naturally existing bacteria in Antarctica (White *et al.*, 2000). Bacillus have also been reported in several literatures from Antarctic soil (Encheva *et al.*, 2013; Logan *et al.*, 2000; White *et al.*, 2000).

CONCLUSION

Our result clearly shows that bacillus spp are present in the soil samples of Antarctica as reported by other researchers. Elaborate study need to be carried out on the potentials of the new isolated bacteria, it ability to degrade pollutants like petroleum hydrocarbons or halogenated toxic compounds. Other biotechnological applications like enzyme production, production of biofuels, production of antimicrobial agents should be explored.

Aknowledgement

We thank the Antarctica Research team of University Teknologi Malaysia and Bauchi State Government of Nigeria for the necessary Support.

REFERENCES

Boesenberg-Smith, K. A., Pessarakli, M. M. and Wolk, D. M. (2012). Assessment of DNA yield and purity: an overlooked detail of PCR troubleshooting. *Clinical Microbiology Newsletter*. 34(1), 1-6.

D'Amico, S., Marx, J.-C., Gerday, C. and Feller, G. (2003). Activity-stability relationships in extremophilic

enzymes. *Journal of Biological Chemistry*. 278(10), 7891-7896.

Encheva, M., Zaharieva, N., Kenarova, A., Chipev, N., Chipeva, V., Hristova, P., Ivanova, I. and Moncheva, P. (2013). Abundance and activity of soil actinomycetes from Livingston Island, Antarctica. *Bulgarian J Agric Sci*. 19(Suppl 2), 68Ā71.

Feller, G. and Gerday, C. (2003). Psychrophilic enzymes: hot topics in cold adaptation. *Nature reviews microbiology*. 1(3), 200-208.

Ganzert, L., Lipski, A., Hubberten, H.-W. and Wagner, D. (2011). The impact of different soil parameters on the community structure of dominant bacteria from nine different soils located on Livingston Island, South Shetland Archipelago, Antarctica. *FEMS microbiology ecology*. 76(3), 476-491.

Hall, T., Biosciences, I. and Carlsbad, C. (2011). BioEdit: an important software for molecular biology. *GERF Bull Biosci*. 2(1), 60-61.

Haruna, I. (2019). A preliminary investigations of novel dehalogenase producing bacteria from antarctic psychrotropic Bacillus sp. IH1. *Access Microbiology*. 1(1A), 13.

He, F. (2011). E. coli genomic DNA extraction. *Bio-protocol*. e97-e97.

Kharsany, A., Hoosen, A., Kiepiela, P., Kirby, R. and Sturm, A. (1999). Phylogenetic analysis of Calymmatobacterium granulomatis based on 16S rRNA gene sequences. *Journal of medical microbiology*. 48(9), 841-847.

Kim, M., Morrison, M. and Yu, Z. (2011). Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. *Journal of microbiological methods*. 84(1), 81-87.

Logan, N., Lebbe, L., Hoste, B., Goris, J., Forsyth, G., Heyndrickx, M., Murray, B., Syme, N., Wynn-Williams, D. and De Vos, P. (2000). Aerobic endospore-forming bacteria from geothermal environments in northern Victoria Land, Antarctica, and Candlemas Island, South Sandwich archipelago, with the proposal of Bacillus fumarioli sp. nov. *International Journal of Systematic and Evolutionary Microbiology*. 50(5), 1741-1753.

Madden, T. (2003). The BLAST sequence analysis tool. *The NCBI handbook*.

Madden, T. (2013). The BLAST sequence analysis tool. *The NCBI handbook*. 2, 425-436.

Moyer, C. L. and Morita, R. Y. (2007). Psychrophiles and psychrotrophs. *Encyclopedia of life sciences*. 1(6).

Nichols, D., Bowman, J., Sanderson, K., Nichols, C. M., Lewis, T., McMeekin, T. and Nichols, P. D. (1999). Developments with Antarctic microorganisms: culture collections, bioactivity screening, taxonomy, PUFA production and cold-adapted enzymes. *Current Opinion in Biotechnology*. 10(3), 240-246.

Radjasa, O. K., Urakawa, H., Kita-Tsukamoto, K. and Ohwada, K. (2001). Characterization of psychrotrophic bacteria in the surface and deep-sea waters from the northwestern Pacific Ocean based on 16S ribosomal DNA analysis. *Marine Biotechnology*. 3(5), 454-462.

Russell, N. J. (2006). Antarctic microorganisms: coming in from the cold. *Culture*. 27(965), e989.

Sjöling, S. and Cowan, D. A. (2003). High 16S rDNA bacterial diversity in glacial meltwater lake sediment,

Bratina Island, Antarctica. *Extremophiles*. 7(4), 275-282.

White, P. L., Wynn-Williams, D. D. and Russell, N. J. (2000). Diversity of thermal responses of lipid composition in the membranes of the dominant culturable members of an Antarctic fellfield soil bacterial community. *Antarctic Science*. 12(3), 386-393.

Yergeau, E., Schoondermark-Stolk, S. A., Brodie, E. L., Déjean, S., DeSantis, T. Z., Gonçalves, O., Piceno, Y. M., Andersen, G. L. and Kowalchuk, G. A. (2009). Environmental microarray analyses of Antarctic soil microbial communities. *The ISME journal*. 3(3), 340-351.