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Isolation and Identification of Helicase from *Anoxybacillus sp* for DNA amplification

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Abstract

Helicase-producing thermophilic microorganisms were successfully isolated from water samples collected from hot springs in the from Ulu Legong Hot spring, Kedah, Malaysia and were identified up to genus level. The light and scanning microscopy technique were used to identify the morphology of the isolate. Chromosomal DNA from the organism was isolated and used to amplify 16S rRNA and UvrD gene fragments. The gene was amplified by a set of universal primers (F_UNI16S and R_UNI16S). The phylogenetic tree, homological analysis, and detailed comparison of the sequences showed that 16S rRNA gene sequence of the isolate had closest similarities with *Anoxybacillus sp.* Isolates gave PCR fragments of 2149bp which represent the UvrD gene and 1500bp which represent the 16S rRNA respectively of *Anoxybacillus. Anoxybacillus sp* was successfully isolated and identified by using 16S rRNA gene sequence analysis with UvrD gene.

Keywords: Helicase, Anoxybacillus sp., hot spring and 16S rRNA

1. Introduction

Recently, nucleic acid amplification, in particular PCR for identification of pathogenic strains with high sensitivity and specificity was reported (Belanger et al., 2003). However, PCR assay requires expensive instrumentation and well-trained personnel for operation. Since easy to adapt test is developed for accurate identification of pathogenic strains in a timely fashion which is highly desirable for hospital cost containment,

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patient management and prompt epidemiological interventions. DNA helicases catalyze the conversion of double stranded DNAs into single stranded forms, making the bases accessible to various DNA metabolic processes, i.e., replication, recombination, and repair. Helicase-dependent amplification (HDA) is a unique isothermal nucleic acid amplification technique that relies on the use of a DNA helicase enzyme to unwind double-stranded DNA and RNA-DNA hybrid. HDA is an isothermal *in vitro* DNA amplification method based upon the coordinated actions of helicases to separate double-stranded DNA and DNA polymerases to synthesize DNA. Helicases also function in other cellular processes where double-stranded DNA must be separated, including DNA repair and transcription. Nucleic acid amplification through HDA is very robust, and the platform can be applied to both DNA and RNA pathogen amplification (An et al., 2005; Goldmeyer et al., 2007). The current study was undertaken to isolate and identify helicase producing bacteria from local hot spring which is able to grow at elevated temperature.

2. Experimental Procedure

2.1. Isolation and identification of microorganisms

Nutrient broth and nutrient agar were used for isolation of thermophilic microorganism from the hot spring. One mL sample from of each waters, biomats, and sediments sample will be added to nutrient broth (10 mL) and incubated in water bath at 60^{o}C for 7 days. Growth was followed by measuring the increase in turbidity at 600 nm. Then, the culture was streaked onto a nutrient agar plate. Isolation of pure culture was done by using spread plate method and streak plate method.

2.2. Characterization and identification of the isolates

2.2.1. Morphological Studies

Morphological properties were investigated by using 18 hour old bacterial cultures growing on thermus agar plates. These included the wet mount preparations using light microscope Gram staining to confirm Gram reaction. Light micrograph and scanning electron microscopy were performed to identify the ultrastructure of the bacteria.

2.3. Identification of Helicase.

2.3.1. Polymerase chain reaction

Anoxybacillus sp. were subjected to Multiplex PCR for helicase gene and 16SrRNA gene identification. Amplification of UvrD helicase gene and 16SrRNA gene fragments was performed with a thermocycler (Mastercycler, Eppendorf) using a standard PCR reaction mixture and a temperature programme that is described in Table 1. The reaction mixture was set up on ice as follows: 5.0 µl 10x PCR Buffer contains Tris.Cl,KCl, $(NH_4)_2SO_4$,15mM $MgCl_2$, stabilizers with ph of 8.7 (20°C), 10 µl 5x Q-solution ,19.75 µl nucleus free water , 1.0 µl dNTP(10mM), 1.0 µl Primer F_UNI16S (10pmol/µl), 1.0 µl Primer R_UNI16S (10pmol/µl), 1.0 µl Primer Anoxy_F(10pmol/µl) and 1.0 µl Primer Anoxy _R(10pmol/µl),0.25 µl Taq polymerase and 1.0 µl DNA template(50ng/ µl) with 50 µl final volume. The PCR program was initial denaturation 3 minutes at 94°C, 35 cycles: 30 sec at 94°C; 30 sec at 60°C; 1 minute at 72°C; followed by a single period at 72°C for 10 minutes.

2.3.2. Agarose Gel Electrophoresis

DNA fragments were analysed and separated via 1% agarose gel electrophoresis. The DNA samples and a

DNA ladder (1kb marker) were stained with DNA loading dye , applied to the gel and separated at 80V for 90minutes. The DNA was visualized using an ultraviolet transilluminator and analysed by size.

3. Results and Discussion

The remarkable increase of newly isolated extremophilic microorganisms, analysis of their genomes and investigations of their enzymes by academic and industrial laboratories demonstrate the great potential of extremophiles in industrial biotechnology (Egorova and Antranikian, 2005). Due to the exceptional features of thermophile, their potential applications in industry and medical biotechnology are far reaching, ranging from production bio-battery to production of substances of medical use. Hence, the current study was undertaken to isolate and identify *Anoxybacillus sp* with helicase to use for development of low setting diagnostic kit for commercially marginalized third world community. *Anoxybacillus sp* was isolated from from Ulu Legong Hot spring, Kedah, Malaysia. Morphological observation of *Anoxybacillus sp* under light and scanning microscopy showed that the cell is a long rod shaped bacteria with Gram positive (Figure 1a & b). Table 1 shows the primer and PCR condition used for the detection of 16S rRNA gene of isolates and UvrD gene. 16S rRNA gene amplified with F_UNI16S and R_UNI16S primers from *Anoxybacillus sp* isolate was observed on agarose gel electrophoresis. The result showed two amplicon bands with the size of 2149bp which represent the uvrD gene and 1500bp which represent the 16S rRNA of *Anoxybacillus sp* respectively (Figure 2).

Table 1. Primers and PCR conditions used for detection of uvrD gene

	Primers Used	**PCR conditions**	**No. of cycles**
UvrD	Anoxy_F 5'- GAC GAATTC TTG ACG TTA CAC AAA GGA – 3' EcoR1 Anoxy _R 5'- TAT CTCGAGTTA GAC AAG GCT TTT CGC - 3' Xho1	Initial Denaturation 94°C – 3min Denaturation 94°C – 30 sec Annealing 60°C – 30sec Extension 72°C – 1min Final Extension 72°C – 10 min Hold 5°C – ∞	35
16SrRNA	F_UNI16S 5'- AGA GTT TGA TCC TGG CTC AG – 3' F_UNI16S 5'- AAG GAG GTG ATC CAG CC -3'	Initial Denaturation 94°C – 3min Denaturation 94°C – 30 sec Annealing 60°C – 30sec Extension 72°C – 1min Final Extension 72°C – 10 min Hold 5°C – ∞	35

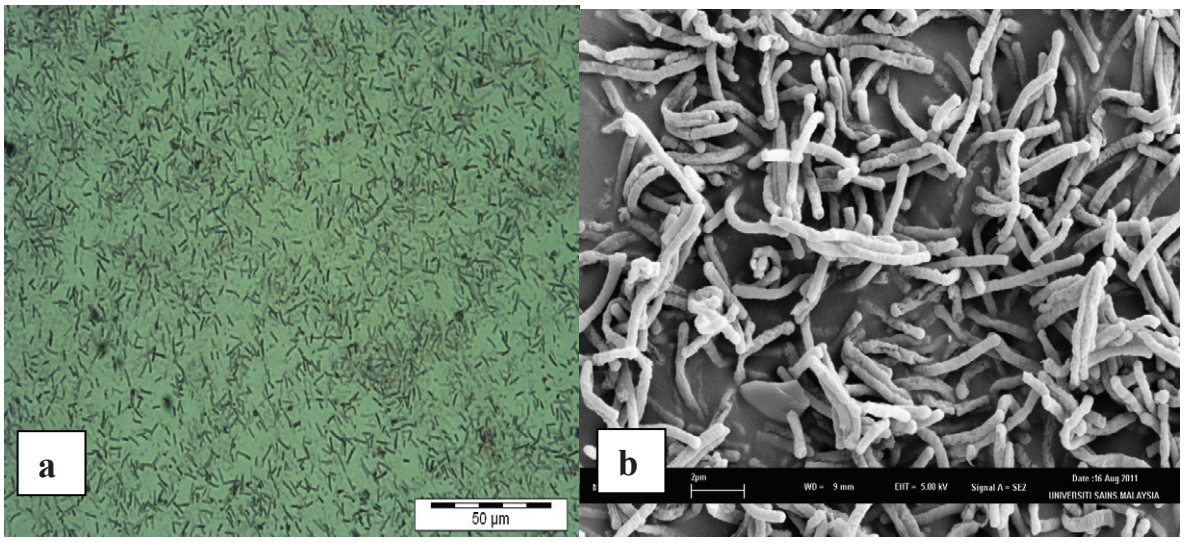

Fig. 1. (a) Morphology of the cells under Light Microscope with 40X amplification.; **(b)** SEM picture of *Anoxybacillus sp*.

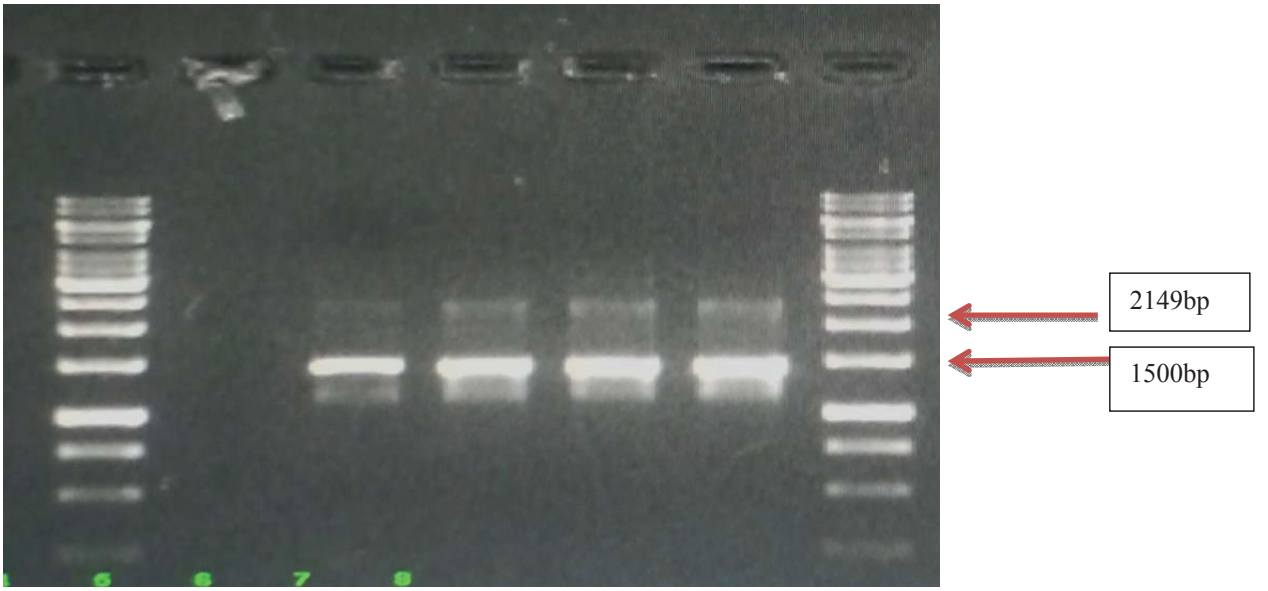

Fig. 2. Amplification of UvrD gene and 16S rRNA gene from Anoxybacillus sp .isolates gave PCR fragments of 2149bp which represent the uvrD gene and 1500bp which represent the 16S rRNA consequently of Anoxybacillus sp. using multiplex PCR. Lanes: M, 1kb DNA ladder; 1 until 6,the Anoxybacillus isolates with different gradient temperature.

Helicase is an enzyme that plays essential roles in nearly all DNA metabolic transactions and has been implicated in a variety of human genetic disorders. UvrD is a highly conserved helicase involved in DNA repair and it plays a critical role in maintaining genomic stability and facilitating DNA lesion repair in many prokaryotic species as it also unwinds and separates the two strands of the DNA double helix. Helicases are

essential during DNA replication because they separate double-stranded DNA into single strands allowing each strand to be copied. Based on the results of this study, helicase (UvrD gene) was successfully isolated from *Anoxybacillus*. After transfer the UvrD gene to an appropriate vector, helicase could be produced in house for various biotechnological purposes especially for the development diagnostic kit. The major medical applications of helicase are in development of diagnostic tests for low-resource settings aimed at low-income countries.

4. Conclusion

In Conclusion, based on the gel image results of this study, the isolated strains showed presence of helicase and *Anoxybacillus sp.* was successfully isolated from geothermal water with Gram – positive rods and aerobic characteristics.

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References

[1] An L, Tang W, Ranalli T.A, Kim H.J, Wytiaz J, Kong H. Characterization of a thermostable UvrD helicase and its participation in helicase-dependent amplification. *J Biol Chem* 2005;**32**:28952-28958.

[2] Belanger S.D, Boissinot M, Clairoux N, Picard F.J, Bergeron M.G. Rapid detection of Clostridium difficile in feces by real-time PCR. *J Clin Microbiol* 2003;**2**:730-734.

[3] Biswas, E.E, Robert G, Biswas,S.B. A novel human hexameric DNA helicase: expression, purification and characterization. *Nucleic Acids Research* 2001;**29**: 1733-1740.

[4] Biswas,E.E, Ewing,C.M, Biswas,S.B. Characterization of the DNA-dependent ATPase and a DNA unwinding activity associated with the yeast DNA polymerase αcomplex. *Biochem* 1993;**32**: 3020–3026.

[5] Bradford, M. M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem* 1976;**72**: 248-254.

[6] Goldmeyer J, Kong H, Tang W. Development of a novel one-tube isothermal reverse transcription thermophilic helicase-dependent amplification platform for rapid RNA detection. *J Mol Diagn* 2007;**5**:639-644.

[7] Adiguzel A, Ozkan H, Baris O, Inan K, Gulluce M, Sahin.F. Identification and characterization of thermophilic bacteria from hot springs in Turkey. *J.Microbiol* 2009;**79**:321-328.

[8] Zuridah H, Norazwin N, Aisyah M.S, Fakhruzzaman M.N.A, Zeenathul N.A. Identification of lipase producing thermophilic bacteria. *J.Microbiol* 2011;**21**:3569-3573.

[9] Canakci S, Kacagan M, Inan K, Belduz A.O, Saha B.C. Cloning, purification, and characterization of a thermostable α-L-arabinofuranosidase from *Anoxybacillus kestanbolensis* AC26Sari. *App.Microbiol.Biotechnol* 2008;**81**:61-68.

[10] Ay F, Karaoglu H, Inan K, Canakci S, Belduz O.A. Cloning, purification and characterization of a thermostable carboxylesterase form *Anoxybacillus sp*. PDF1. *Protein.Exp.Purification* 2011;**80**:74-79.

[11] Che`ne P . PcrA/UvrD/Rep DNA helicases in bacterial genomes. *Biochem.Sys.Eco* 2008;**36**:101-109.

[12] Egorova K, Antranikian G. Industrial relevance of thermophilic Archaea. *Curr Opin Microbiol* 2005;**8**: 649–655.