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Psychrophilic Lipase from Arctic Bacterium

Zakiah Ramle and Rashidah Abdul Rahim*

School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia

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Abstract: A lipase producer psychrophilic microorganism isolated from Arctic sample was studied. The genomic DNA of the isolate was extracted using modified CTAB method. Identification of the isolate by morphological and 16S rRNA sequence analysis revealed that the isolate is closely related to *Arthrobacter gangotriensis* (97% similarity). *A. gangotriensis* was determined as positive lipase producer based on the plate screening using specific and sensitive plate assay of Rhodamine B. The PCR result using *Arthrobacter* sp.'s full lipase gene sequence as the template primers emphasised a possible lipase gene at 900 bp band size. The gene is further cloned in a suitable vector system for expression of lipase.

Keywords: Arctic, Psychrophiles, Lipase

INTRODUCTION

Psychrophiles are defined as organisms having an optimal temperature for growth at 15°C or lower with a maximum temperature for growth at about 20°C (Morita 1975). They produce enzymes which are adapted to work at low temperature. Obligate psychrophiles are organisms that prefer temperature close to zero for growth. The term psychrotrophic used refers to "organisms previously known as facultative psychrophiles" or psychrotolerant, with maximum temperature above 20°C (Gounot 1986). Previous studies have it have shown that cold-adapted microorganisms are potential sources of cold-active enzymes that can exhibit higher catalytic activity at low temperature (Gerday *et al.* 2000; Giudice 2006).

One of the enzymes produced by psychrophilic microorganisms includes lipases. Triacylglycerol hydrolases (E.C.3.1.1.3), also recognised by its common name as lipases, are enzymes that catalyse the hydrolysis of long-chain acylglycerols in aqueous emulsions (Svetlana 2010). Lipases normally catalyse heterogenous reactions at the interface of water and insoluble systems (Eltaweel

*Corresponding author: rshidah@usm.my

et al. 2005). The versatility of these enzymes with multiple approaches towards its utilisation contributes to high interest in lipases. With broad range of substrate and reaction specificity and the ability to catalyse reaction in the absence of a cofactor, lipases' largest industrial use still remains the hydrolysis of fats and oils (Leonov 2010).

MATERIALS AND METHODS

Isolation of Psychrophilic Bacterium

Psychrophilic bacterium used in this study was obtained from Arctic soil sample collected by the Polar Research Group of Universiti Sains Malaysia (USM) Pulau Pinang. Sampling took place in August 2011, on the northern coast of Hornsund, Wedel Jarlsberg Land, West Spitsbergen (°00'04"N, 15°33'37"E).

Temperature Studies

Isolate was incubated at three different temperatures of 4°C, 27±2°C, and 37°C to test whether the isolate fits the definition of an obligate psychrophile or facultative psychrophile (psychrotolerant).

Identification of Psychrophilic Bacterium

Morphological and molecular approaches such as Gram's staining and 16S rRNA sequencing were conducted to identify isolate. DNA extraction following modified CTAB method was conducted prior to 16S rRNA sequencing. 16S' universal primers with sequence of 27F(5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R(5'-TAC GGY TAC CTT ACG ACT T-3') were used in gene amplification. Purified PCR product was sent to First Base Laboratories Sdn. Bhd. for sequencing and the sequence was applied to Basic Local Alignment Search Tool (BLAST).

Screening of Lipolytic Activity

Nutrient agar supplemented with 0.5% (v/v) of olive oil and 0.01% (w/v) of Rhodamine B and 0.5% (v/v) tributyrin were used to test for lipolytic activity. Formation of halo zone surrounding colony showed lipolytic activity. The best lipase producer was chosen to be used in further studies.

Cloning of Lipase Gene

A primer was first designed based on full lipase gene's sequence obtained from NCBI. The primer with sequence P3F(5'-GCA GCA TAT GAG CGT GAG TG-3') and P3R(CGG ATA ACC CAC GCA ACA AA-3') was used in PCR. Possible lipase gene at expected size observed via gel electrophoresis was further purified and cloned into a suitable vector system for expression of lipase.

RESULTS AND DISCUSSION

A total of three isolates were obtained originally labelled as ARB 1A, ARB 1B, and ARC 8B. Table 1 shows the plates for ARB 1B observed in temperature studies where incubation took place in three different temperatures 4°C, 27±2°C, and 37°C. Based on the plates observed, a conclusion was made that all isolates were able to grow at room temperature thus they were classed as psychrotolerant or facultative psychrophiles microorganisms. Obligate psychrophiles and psychrotrophs or psychrotolerant both have the ability to grow at or close to zero, but they differ in the optimum and upper temperature limits for growth in which psychrophiles are lower (Russell *et al.* 1990). In identification of isolate via morphological approach, both ARB 1A and ARC 8B were identified as rod-shaped Gram negative bacteria while ARB 1B was a coccus-shaped Gram positive bacteria (figure not shown). Based on 16S rRNA sequencing, it was proven that all three isolates did not belong to the same species. Table 2 shows the BLAST results of 16S rRNA sequencing.

With 99% similarities, ARB 1A was determined as *Pseudomonas gessardii*, while ARC 8B was *Janthinobacterium lividum*. The ARB 1B shared 97% similarities with *A. gangotriensis*. These results were tally with Gram's staining as *Pseudomonas* sp. and *Janthinobacterium* sp. are both Gram negative bacteria and *Arthrobacter* sp. is Gram positive.

In choosing the best lipase producer for further studies, tributyrin test was conducted. Formation of clear zone surrounding colonies indicated lipolytic activity. Figure 1 shows formation of clear zone on tributyrin agar plate. Tributyrin agar is often used as preliminary screening of lipase activity (Glogauer *et al.* 2011). Tributyrin agar was prepared as emulsions that will make the agar appear opaque. When a lipase-positive organism was streaked, clear zones will be formed around the growth as evidence of lipolytic activity. The organism is termed lipase-negative if no clear zones observed (Leboffe *et al.* 2011). *Arthrobacter* sp. was chosen for further study based on this test.

A lipase primer with forward and reverse's sequences P3F(5'-GCA GGA TAT GAG CGT GAG TG-3') and P3R(5'-CGG ATA ACC CAC GGA ACA AA-3') was designed based on *Arthrobacter* sp. HW08 GDSL-like lipase gene's full sequence obtained from NCBI as a template. The primer was used in lipase gene amplification (PCR). Figure 2 shows the gel electrophoresis of PCR product using the designed primer with annealing temperature of 54°C.

Lane 1 is by default the lane for Gene ruler + 1kb DNA ladder. In Figure 2, lane 2 and 3 was the PCR product. Both replicates for PCR product showed band sizes of ~900bp in lane 2 and 3. The bands were excised and further purified. Figure 3 shows the gel electrophoresis of purified PCR product also with the same expected size. The bands were consistent in both replicates and they showed the expected band size of approximately 900 bp. Purified PCR product will then be digested and transformed into suitable vector system for lipase expression.

Table 1: Plates observed for ARB 1B (*Arthrobacter gangotriensis)* at three different incubation temperatures. Colonies were seen growing at both 4°C and 27±2°C which deduced that the isolate is a facultative psychrophile (psychrotrophs). Facultative psychrophiles have higher maximum temperature for growth which can exceed 20°C.

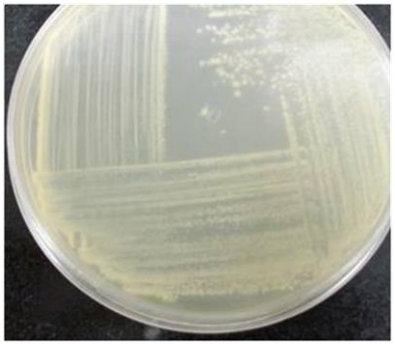
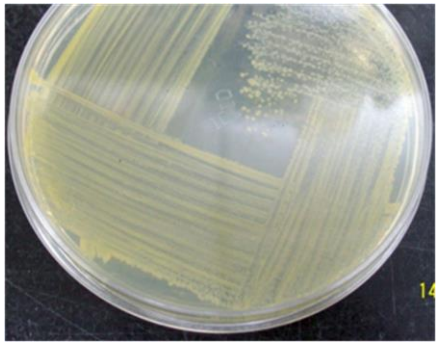
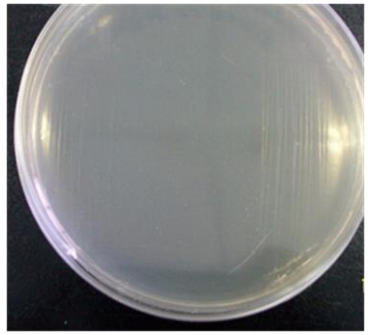
Temperature (°C)	4	27±2	37
ARB 1B			

Table 2: BLAST results of 16S rRNA sequencing for ARB 1A, ARB 1B, and ARC 8B.

Isolate	Description	Max score	Total score	Query coverage (%)	E value	Max identity (%)
ARB 1A	*Pseudomonas gessardii* strain CIP 105469 16S ribosomal RNA, partial sequence	2402	2402	97	0.0	99
ARB 1B	*Arthrobacter gangotriensis* strain Lz1Y 16S ribosomal RNA partial sequence	2263	2263	98	0.0	97
ARC 8B	*Janthinobacterium lividum* strain DSM 1522 16S ribosomal RNA, partial sequence	1996	1996	93	0.0	99

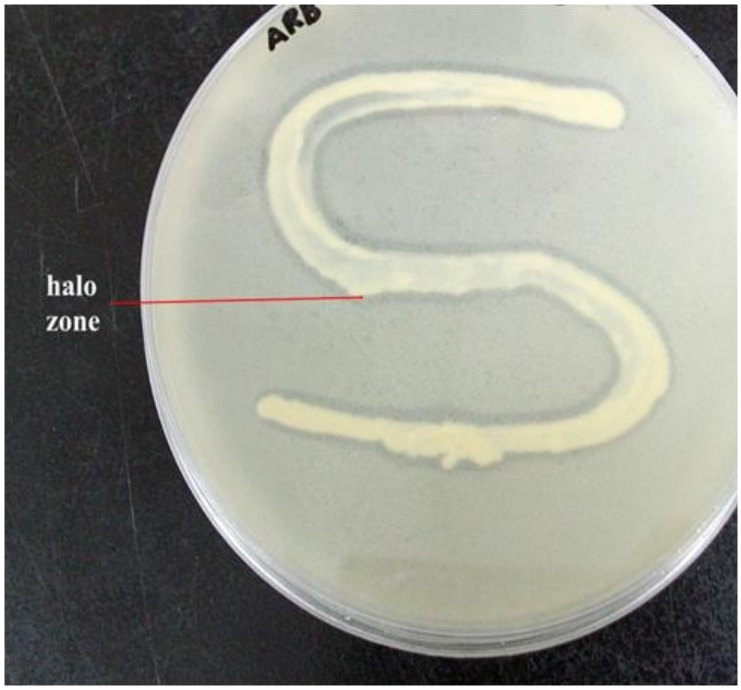


Figure 1: Formation of halo zone on tributyrin screening plate.

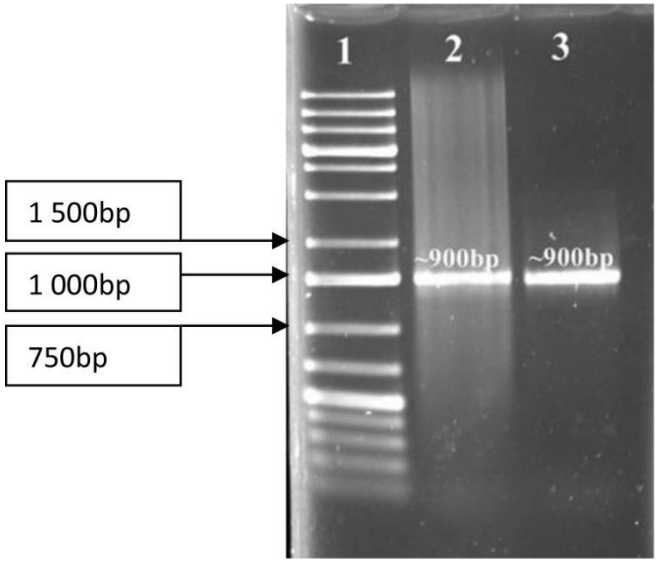


Figure 2: Gel electrophoresis of PCR product using specifically designed primer to amplify lipase gene. Lane 1: gene ruler + 1kb DNA ladder; Lane 2–3: PCR product.

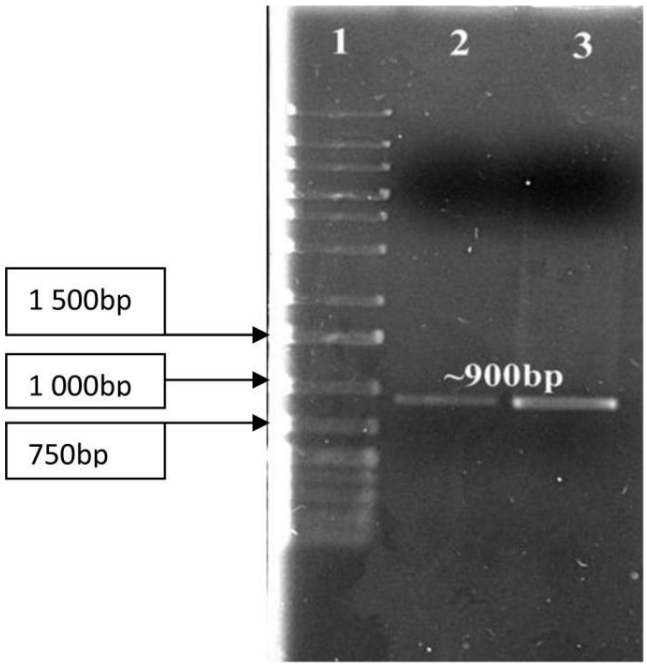


Figure 3: Gel electrophoresis of purified PCR product with bands showing expected size of ~900 bp. Lane 1: gene ruler + 1kb DNA ladder; Lane 2–3: purified PCR product.

CONCLUSION

A coccus-shaped Gram positive psychrotrophic Arctic bacterium was successfully isolated and was identified as *A. gangotriensis.* This isolate was determined as positive lipase producer based on the preliminary screening test for lipolytic activity. The lipase gene was amplified and will be cloned and expressed in appropriate vector to further characterise the enzyme. Enzyme that is of high interest is the one that has distinct characters that can tolerate wide pH range, exhibit good stability in extreme temperature, maintain active in various organic solvents and can be produced in bulk production (Bornscheuer *et al.* 2002).

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