Lipase from the Antarctic microorganism

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Abstract

Cold-active enzymes are usually characterised by a higher specific activity at low temperature when compared to their mesophilic counterparts and offer a high potential in numerous biotechnological applications. In this study, an obligate psychrophilic microorganism was isolated from water at Davis Station, Antarctica. The isolate named PI A was grown on screening plate to screen for extracellular lipase. It was shown to hydrolyse the oil substrate by forming the halo zone. In order to isolate the lipase gene, genomic library was done using the genomic DNA. The genomic DNA which had been successfully extracted by using phenol-chloroform extraction with the modification of CTAB (cetyltrimethylammonium bromide) method from plant was digested with *Sau3A*1 to produce small fragments of DNA. The recombinant plasmid was transformed into competent *E.coli*. The recombinant *E.coli* harboring a putative lipase gene was detected due to its ability to hydrolyse nutrient agar medium with tributytrin plate containing ampicillin at 5°C. A few analyses were carried out to detect the insertion of the DNA fragment in the plasmid and the size of the lipase gene that had been cloned.

Keywords: psychrophile, lipolytic activity, genomic, plasmid

INTRODUCTION

Lipases (EC 3.1.1.3) are a class of hydrolases that are primarily responsible for the hydrolysis of triacylglycerides at the interface between the insoluble substrate and water (Snellman *et al.*, 2002). In addition to their biological significance, lipases hold tremendous potential for exploitation in biotechnology. They posses the unique feature of acting at the aqueous and non – aqueous interface which distinguishes them from esterases (Verger, 1997; Schmidt and Verger, 1998).

The psychrophilic microorganisms have the largest distribution on earth if one considers the extent of area where temperature remains permanently below 10°C including the deep-sea waters, mountains and polar regions. Life in low temperature environments requires physiological and biochemical adaptations of microorganisms. Psychrophilic organisms grow fastest at temperature of 15°C or lower, but cannot survive at temperature above 20°C whereas; psychrotolerants grow well at temperatures close to the freezing point of water, but have fastest growth rates above 20°C up to 40°C (Madigan *et al.*, 2003).

Psychrophilic organisms produce enzymes that can function in cold environment and simply denature at high temperature. The cold-active enzymes have a more flexible structure to undergo the conformational changes necessary for catalysis with a lower energy demand (Arpigny *et al.*, 1997).

Low temperature-active enzymes have recently received increasing attention because of their

relevance for both basic and applied research. Cold-active enzymes might offer novel opportunities for biotechnological exploitation based on their high catalytic activity at low temperatures, low thermostability and unusual specificities (Russell, 2000). These properties are of interest in diverse fields such as detergents, textile and food industry, bioremediation and biocatalysts under low water conditions (Choo *et al.*, 1998). In addition, enzymes isolated from organism native to cold environments generally exhibit higher catalytic efficiency at low temperature and greater thermosensitivity than their mesophilic counterparts (Gerday *et al.*, 1997) and is particularly useful to investigate the structure-function relationship using their mesophilic and themophilic counterparts for comparison.

In this research, the targeted gene will be recovered from the genomic library and further analysis will be carried out to determine the functional gene.

MATERIALS AND METHODS

Strain and culture conditions

Psychrophilic microorganism named PI A, a lipase-producing microorganism was isolated from fresh water sample at Davis Station, Antarctica. The cultures were maintained at 5°C, on nutrient agar.

Lipase production

The lipolytic activity was detected by the development of clear halos surrounding the colonies on three kinds of agar plates as follows. The nutrient agar medium supplemented with tributyrin medium (1% v/v), or palm oil medium (0.5% v/v) or Rhodomine B medium (1% v/v olive oil and 10ml of Rhodomine B stock 0.01% w/v) (Kouker & Jagger, 1987).The preparation of culture media was done at pH 7.0 and the mixture was well homogenized by a mixer. Cultures were incubated for 7-10 days for tributyrin medium and 2-3 weeks for palm oil and Rhodomine B medium at 5°C. Lipase production on Rhodomine B medium was monitored by fluorescence with UV light at 350nm.

Genomic DNA extraction

Standard protocols of Sambrook *et al.*, (1989) were used for DNA manipulation. Genomic DNA (gDNA) from PI A was isolated directly from the cells grown in nutrient broth using phenol-chloroform extraction with the modification of CTAB (cetyltrimethylammonium bromide) method from plant.

Partial Digestion

Partial digestion of genomic DNA was performed by serial dilution technique to obtain the optimum enzyme concentration in order to generate a certain range of fragment size. Serial dilutions were prepared on ice using *Sau3A*1 restriction enzyme. The digestion reactions were incubated at 37°C for 15 minutes and the reactions were stopped by incubating at 65°C for 20 minutes.

Construction of plasmid based library

The partially digested genomic DNA fragments were ligated with *BamH*1 digested pUC18 and transform into *E.coli* JM109. The genomic library was screened on agar plate containing ampicillin, IPTG and X-gal. The recombinants were screened randomly to analyse the size of insert being cloned.

Screening gene of interest
In order to obtain the gene of interest, the recombinant library was screened on nutrient agar plate containing tributyrin.

RESULTS AND DISCUSSIONS

Characterisation of the strain
The isolated microorganism is a rod with budding shaped and size around 4.2µm. This microorganism could survive a wide range of temperature from 0 °C to 20 °C and has optimum growth temperature at 5°C. According to the definition of Morita (1975), strain PI A was a psychrophile

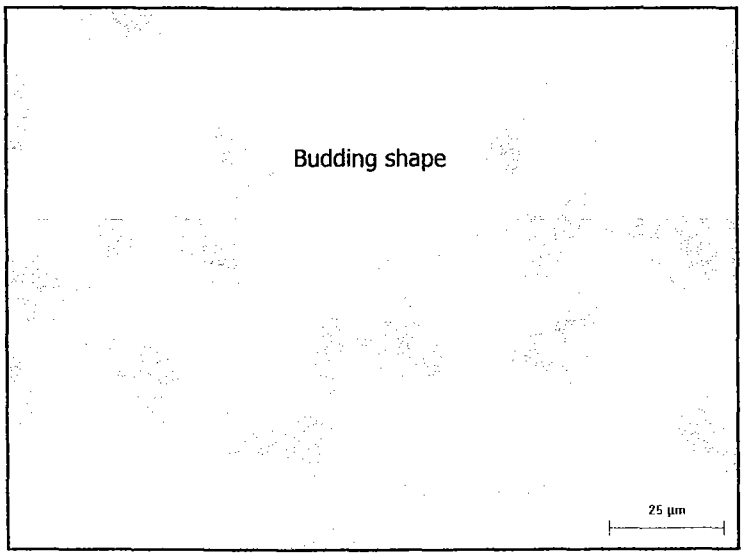

Fig 1: PI A cells grown in nutrient broth as observed under phase contrast microscopy

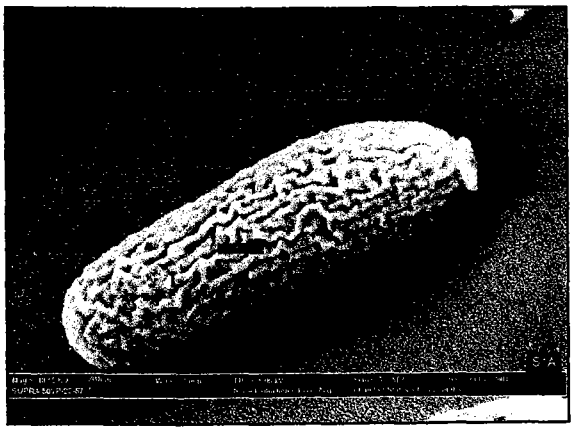

Fig. 2: Scanning electron microscope (SEM) of PI A

Lipase production

The lipase activities were detected through the formation of clear halos surrounding the colonies on the different agar plates as shown below (See Fig. 3, Fig. 4 and Fig. 5).

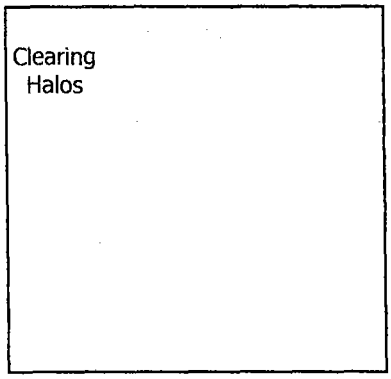

Fig. 3: Hydrolysis zone surrounding the PI A colonies on tributyrin agar plate after 7 days of incubation at 5°C.

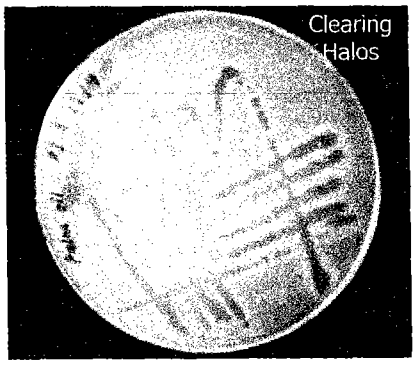

Fig. 4: Hydrolysis zone surrounding the PI A colonies on palm oil agar plate after 7 to 14 days of incubation at 5°C.

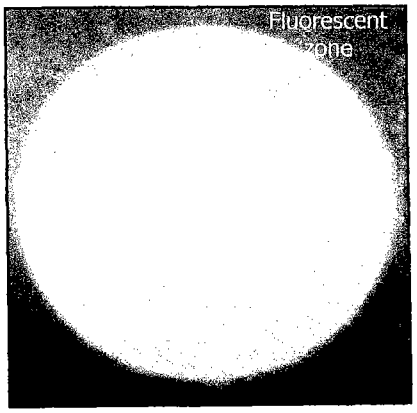

Fig. 5: PI A colonies on nutrient agar with rhodomine B showed orange fluorescent colour under the UV light (Kouker & Jagger, 1987).

Genomic DNA extraction

The genomic DNA was successfully extracted using phenol-choloform extraction with the modification of CTAB (cetyltrimethylammonium bromide) method from plant. Figure below shows gDNA of PI A on the agarose gel (0.7%).

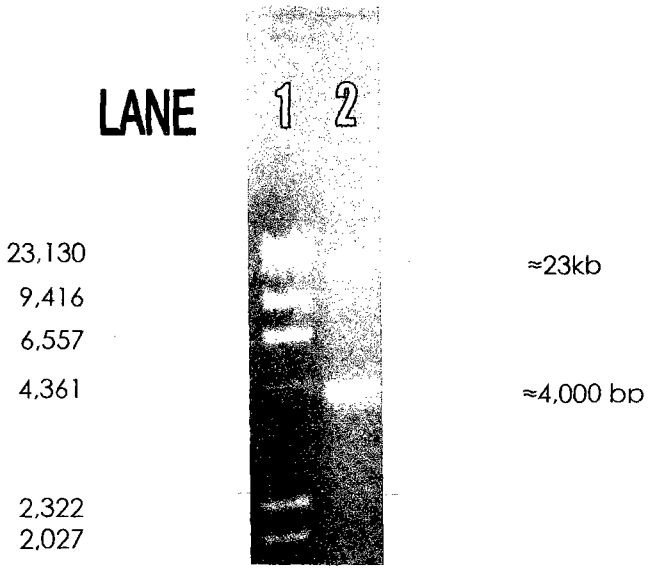

Fig 6: gDNA extraction. Lane 1: Lambda Hind III / DNA Marker and Lane 2: gDNA of PI A

Partial Digestion

PI A gDNA was successfully prepared to partially fragment by partial digestion. Partial digestion of gDNA was performed by serial dilution of restriction digestion reaction mix. The optimum condition produce 2-4 kb fragment was found to be gDNA digested with 10^{-3} dilution factor. Figure below shows the electrophoretic (0.7%) separation of gDNA digested by various concentration of *Sau3A*1 restriction enzyme.

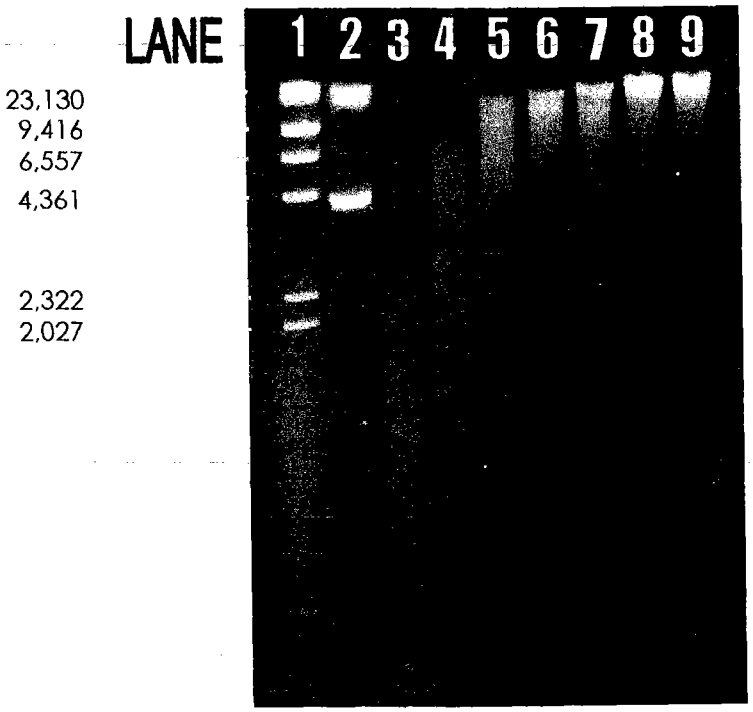

Fig 7: Partial digestion of gDNA by serial dilution method. Lane 1: Lambda Hind III / DNA Marker, Lane 2: gDNA of PI A, Lane 3: gDNA digested with 10^{-1} dilution factor, Lane 4: gDNA digested with 10^{-2} dilution factor, Lane 5: gDNA digested with 10^{-3} dilution factor, Lane 6: gDNA digested with 10^{-4} dilution factor, Lane 7: gDNA digested with 10^{-5} dilution factor, Lane 8: gDNA digested with 10^{-6} dilution factor and Lane 9: gDNA digested with 10^{-7} dilution factor.

Construction of plasmid based library

The genomic library was screened on agar plate containing ampicillin, IPTG and X-gal resulted around 5000 recombinants. Plasmids isolated from the recombinants were randomly selected and digested with *EcoR*1 (See Fig. 8). The inserts size was between 2-4kb.

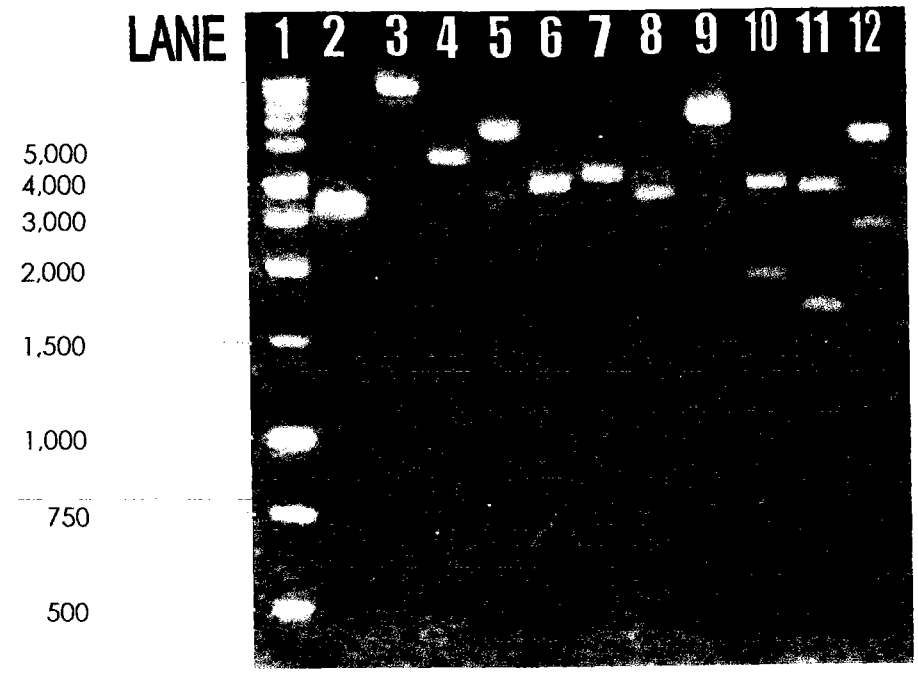

Fig 8: Analysis of inserts on randomly selected plasmid. Lane 1: 1kb Marker, Lane 2: Undigested plasmid, Lane 3 - 12: Digested plasmid with *EcoR*1

Screening gene of interest

Out of 50 colonies, five positive recombinants were observed produced halos of clearing on the tributyrin agar plates after 5 to 7 days of incubation at 15°C indicating the lipase activity. Figure below shows the lipase activities detected on the tributyrin agar plate.

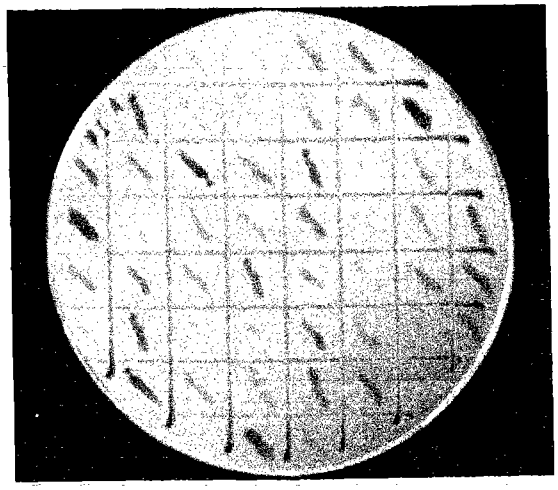

Fig.9: Detection of the lipase activity on the 1% of tributyrin agar plate after 5 to 7 days of incubation at 15°C. The clear zone of lipolysis was formed.

CONCLUSION

In conclusion, the genomic library of lipase - producing microorganism, PIA was successfully constructed using plasmid based vector. The library was used to screen the gene of interest. Further analysis is needed to determine the selected gene.

ACKNOWLEDGEMENTS

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