LIPASE FROM PSYCHROPHILIC MICROORGANISM ISOLATED FROM THE ANTARCTIC FRESH WATER

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Low temperature-active enzymes have recently received increasing attention because of their relevance for both basic and applied research. In biotechnology, novel opportunities might be offered by their catalytic activity at low temperature and, in some cases, unusual specificity.
An obligate psychrophilic microorganism, which hydrolyses lipids at 4°C, was isolated from fresh water at Davis Station, Antarctic. The isolate is a rod with budding shaped, gram-positive bacterium and size around 4.2μm. Phenotypic identification to identify the genus of strain by using morphological studies and biochemical tests was carried out. The isolate grew at 4°C and 15°C for 7 days incubation period. Isolate named PI A was grown on screening plates which contained nutrient agar and lipase substrate such as tributyrin, triolein, palm oil, olive oil and fluorescent assay using Rhodamine B to screen for extracellular lipase. The existence of the halos on the media showed the hydrolytic reaction by lipase activity. Lipase assay using titration was also done to detect the lipase activity. Lipase activity was detected at 1.647 U/ml.

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INTRODUCTION

Most microorganisms isolated from cold environments that are able to grow at or close to the freezing point of water are called psychrophiles. They are defined by an optimal temperature for growth at about 15°C or below, a maximal growth temperature of 20°C and an ability to grow at 0°C (Morita, 1975).

Cold-active microorganisms live at such low temperatures, where most other species cannot grow and to survive they need to produce enzymes able to perform efficiently their catalysis under these extreme environmental conditions. Their products have potential applications in a broad range of industrial, agricultural and medical processes.

The objectives of this work were to identify cold-active lipase producing psychrophilic microorganism and to screen the extracellular lipase by using selective agar medium for lipase activity.

MATERIALS AND METHODS

Organism

Psychrophilic microorganism used in this study was isolated from Antarctic fresh water sample on nutrient agar plates and named as PI A.

Phenotypic identification of PI A

PI A was characterised via conventional morphological observation and biochemical tests.

Screening and lipase production

Different kind of solid medium were used to detect lipase activity which were nutrient agar medium (pH 7) containing peptone (5.0g/L), beef extract (3.0g/L) and agar (12.0 g/L) supplemented with tributyrin medium (1% v/v), or palm oil medium (0.5% v/v) or Rhodomine B medium (Kouker & Jagger, 1987) 1% v/v olive oil and 10ml of Rhodomine B stock 0.01% w/v. 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 4°C. The lipolytic activity was detected through the formation of clear halos around the colonies in different kinds of agar plates as mentioned above at 4°C. Lipase production on Rhodomine B medium was monitored by fluorescence with UV light at 350nm. The agar plates were incubated at 5°C and room temperature (≈24°C). The supernatant that contained lipase enzyme would hydrolyse the substrate and produced halo zone surrounding the holes on the agar plates.

Preparation of crude enzyme extract

The cultures from tributyrin, palm oil and olive oil broth medium were used in preparation of crude enzyme extract. PI A cells were harvested by centrifugation at 6,000 rpm for 15 min at 4°C and the supernatant was assayed for extracellular lipase activity.

Protein Determination

Protein was estimated by the published method of Bradford (1976) with bovine serum albumin as the standard.

Lipase Assay

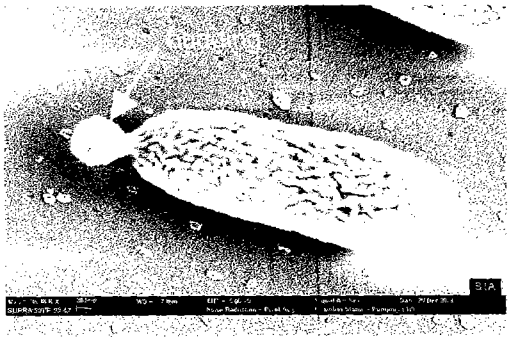
The lipase catalytic activity was measured by titrimetric using 0.02 M NaOH of emulsified substrates to determine the rate of free fatty acid release from tributyrin, palm oil and olive oil (contains 70% triolein).1ml portion of crude extract enzyme was added to 5 ml of emulsion containing 2% (v/v) olive oil, 1% (v/v) gum arabic, 0.10 M Tris and 4 ml of 0.2 M phosphate buffer at pH 7. The assay was carried out at 4°C and room temperature (≈24°C) during 30 minutes incubation. After this time interval, the reaction was stopped by the addition of 15 ml of acetone:ethanol, 1:1 (v/v) and the amount of fatty acids was then titrated using 1% ethanolic phenolphthalein . One unit of lipase activity was defined as the amount of enzyme required to release 1μmol of fatty acid per min under these conditions (Arima *et al.*, 1972).

RESULTS

Phenotypic identification of PI A

Table 1 shows the results of the microbiological characteristics of PI A. The colony appeared to be circular, smooth and convex on nutrient agar. The bacterium is a gram-positive rod with budding shaped, and size around 4.2µm. It was able to hydrolyse tributyrin, palm oil and olive oil.

Morphology of isolated microorganism.



PI A colony viewed with phase-contrast microscopy (magnification: 40000)

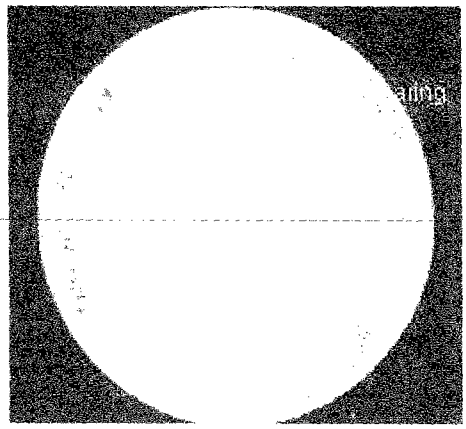
PI A cell viewed under scanning electron microscopic.

Table 1: Phenotype characteristics of the isolate PI A

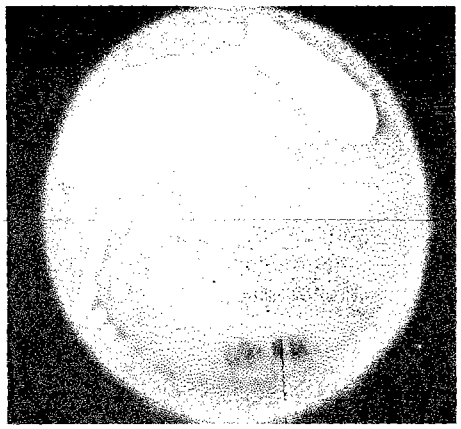
Characteristic			PI A Result
Morphological	Size		3-5µm
	Shape		Rods with budding
	Gram Stain		Positive
Culture	Incubation Period		5-7 days
	Growth Temperature	4°C	+
		12°C	+
		15°C	+
		Room Temperature	-
Hydrolysis Activity	Tributyrin		+
	Palm Oil		+
	Olive Oil		+
Biochemical Test	Citrate test		-
	Urease activity		+
	Indole production		-
	Oxidase test		-
	Catalase Test		+
	Vogue-Proskauer test		+

Tests were repeated 3 times. Notes: (+) positive results; (-) negative results.

Lipase Production



Hydrolisis zone surrounding the PI A colonies on palm oil agar plate after 14 days of incubation at 4°C.



PI A colonies on nutrient agar with rhodomine B showed orange fluorescent colour under the UV light.

CONCLUSION

In conclusion, we were successfully isolated a psychrophilic microorganism, PI A from Antarctica fresh water sample after 7 days of incubation at 4°C. PI A was detected to produce an extracellular lipase activity by showing the clear hydrolysis zones on the lipase selective medium.

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