Psychrophilic Enzymes from the Antarctic Isolates

Rashidah A. R*, Sabrina S, Ainihayati A. R, Shanmugapriya. P, Nazalan N and Razip S

School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
* Corresponding author: rshidah@usm.my

Abstract

In the project, the Antarctic fresh samples will be obtained from different sources. An obligate psychrophilic microorganism from the fresh sample will be isolated by the growth and temperature studies. The selected Antarctic microorganisms will be identified by conventional morphological studies, biochemical tests and molecular characterisation based on the nucleotides sequence in genes 26S rDNA, 16S rRNA and Internal Transcribed Spacer (ITS). The potential industrial enzymes (eg. lipase and protease) and fundamental metabolic (eg. glycolytic pathway) enzymes from the isolated microorganism will be screened for the enzyme studies. Cloning of interest genes will be carried out. The recombinant proteins will be further expressed and purified for the structural studies that will involve protein crystallisation and X-ray crystallography diffraction analysis for the determination of protein structure.

1. Introduction

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).

In order for growth to occur in low temperature environments, all cellular components from membranes and transport systems to intracellular solutes, protein and nucleic acids must adapt to the cold (Cavicchioli *et al.*, 2002). 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).

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). Furthermore, fundamental issues concerning the molecular basis of cold activity and the interplay between flexibility and catalytic efficiency are of important in the study of structure-function relationships in protein.

In this studies two groups of enzymes were involved which were the potential industrial enzymes (eg. lipase and protease) and fundamental metabolic (eg. glycolytic pathway). Extracellular lipases and proteases represent two of the most important groups of extracellular hydrolytic enzymes. Lipases are triacylglycerol ester hydrolases that catalyze the hydrolysis of triglycerides at the interface between the insoluble substrate and water (Snellman *et al.*, 2002). While proteases are enzymes that break peptide bonds between amino acids of proteins (Breker, 1986). The process is called peptide cleavage, a common mechanism of activation or inactivation of enzymes. The use of a molecule of water during the reaction, thus classified them as hydrolases (Plummer, 1987).

A metabolic enzyme is an enzyme involved in a metabolic pathway, inside individual cells. We are focusing on the glycolytic pathway enzymes namely glyceraldehyde 3-phosphate dehydrogenase (GAPDH), triose phosphate isomerase (TPI) and phosphofructokinase (PFK).

The objectives of this work are to isolate the Antarctic psychrophilic microorganisms and to study the potential enzymes produced from the microorganisms.

2. Materials and Methods

2.1 Isolation of an obligate psychrophilic microorganism

At the initial stage of the research, ten strains of microorganisms were isolated at 5°C. All of these strains were isolated from the Antarctic fresh samples. Two strains namely PI A (isolated from freshwater sample) and PI 12 (isolated from seawater) were selected for further studies.

2.2 Identification of microorganism

The microorganisms were characterised via microscopic analysis, biochemical tests and molecular approaches (16S rDNA, 26S rDNA and ITS).

2.3 Production of lipolytic and glycolytic enzymes from a psychrophile

Production of the enzymes were based on their enzymes activity were successfully conducted.

2.4 Cloning and characterisation of lipolytic and glycolitic genes

Lipase and two glycolytic genes, GAPDH and TPI were chosen in the study. The cloning of full length genes from genomic DNA and cDNA templates were carried out for expression study.

2.5 Purification of glycolytic enzyme

The ATP-PFK was purified from the culture broth by simple sequential procedures involving ultrafiltration centrifugation, affinity chromatography, anion exchange chromatography and gel filtration.

3. Results and Discussions

3.1 Isolation and identification of obligate psychrophilic microorganisms

3.1.1 Microscopic analysis and biochemical tests

Under microscopic analysis PI A showed rod with budding shape while PI 12 showed rod-cocci with budding morphology. (See Fig. 1 and Fig. 2)

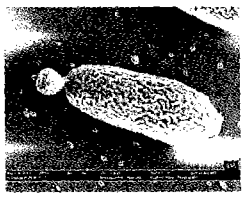


Fig. 1.: PI A cell viewed under scanning electron microscopic

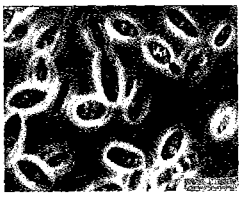


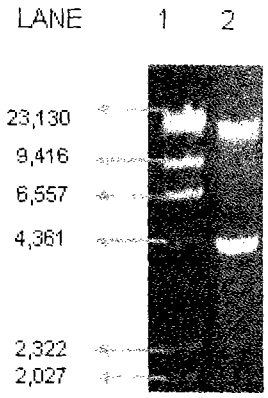
Fig. 2.: PI 12 colonies viewed with phase-contrast microscopy (magnification: 40000)

Characteristic	PI 12	PI A
Cell Diameter (μM)	1.5 – 3.8	2.0 – 4.2
Shape	Rod Cocci with budding	Rod with budding
Colony Formed on plate		
• Shape	Irregular	Round
• Margin	Smooth	Smooth
• Elevasion	Convex	Convex
Incubation Period	5-7 days	5-7 days
Growth Temperature (°C)	0 -20	0-20
Optimum Temperature (°C)	4	4
Urease Test	+	+
Catalase Test	+	+
Oxidase Test	-	-
Nitrate Test	+	-
Growth Medium		
• Luria Broth	+	+
• Define Glucose	+	+
• Protease	+	+
• Starch	-	+
• Gelatine	-	+
• Tributyrin	+	+
Tests Against various antibiotic		
• Ampicillin		
• Chloramphenicol	+	+
• Kanamysin	+	+
	+	+

Table 1.: Biochemical tests data of PI A and PI 12. Notes (+) positive results; (-) negative results

3.1.2 Molecular Approaches

Results for 26S rDNA of PI A and PI 12 revealed 99% sequence identity to *Antarctic yeast CBS 8927* and 100% sequence identity to *Leucosporidium antarcticum, respectively.* While Internal Transcribed Spacer (ITS) results showed 99% sequence identity to *Antarctic yeast CBS 8927* for PI A and 100% sequence identity to *Antarctic yeast CBS 8942* for PI 12. Figures 3-5 showed the Polymerase Chain Reaction (PCR) product produced from the specific primers used in the molecular identification.

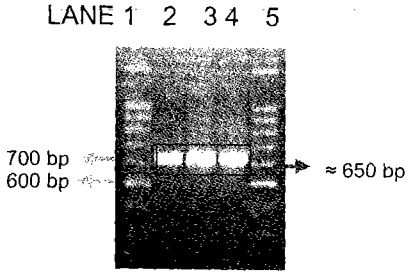


Legends :

Lane 1 : Lambda Hind III / DNA marker

Lane 2 : gDNA of PI A

Fig. 3.: Genomic DNA extraction - gDNA of PI A on the agarose gel (0.7%). The exact size of the genomic was unknown but large bands in the 23kb region

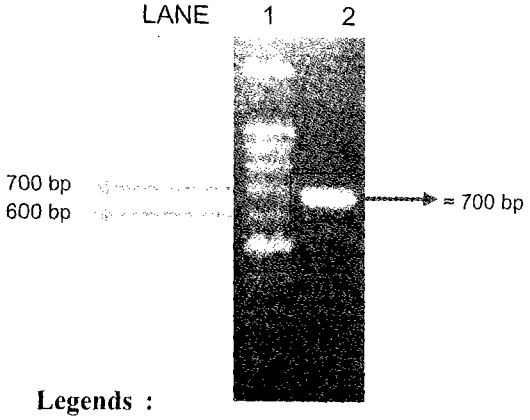


Legends :

Lane 1&5 : 100bp DNA Ladder

Lane 2 - 4 : PCR products

Fig. 4.: PCR product using 26S rDNA forward and reverse primers on the agarose gel (1.0%).



Legends :

Lane 1 : 100bp DNA Ladder

Lane 2 : PCR product

Fig. 5.: PCR product using ITS forward and reverse primers on the agarose gel (1.0%).

3.2 Production of lipase and protease enzymes from a psychrophile

The lipase (See Fig. 6 and Fig. 7) and protease (See Fig. 8) extracellular activities were detected from the isolated psychrophilic microorganism.

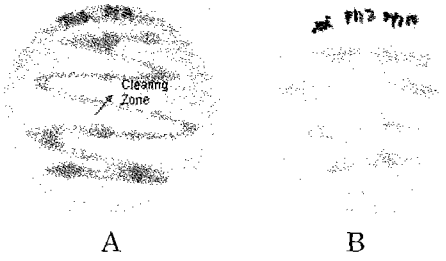


Fig. 6.: Hydrolisis zone surrounding the PI A (photo A) and PI 12 (photo B) colonies on tributyrin agar plate after 7 days incubation at 5°C.

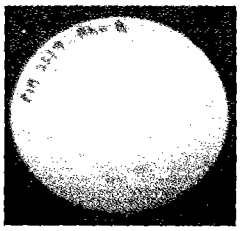


Fig. 7.: PI A colonies on nutrient agar with rhodomine B showed orange fluorescence colour under the UV light (Kouker Jagger, 1987).

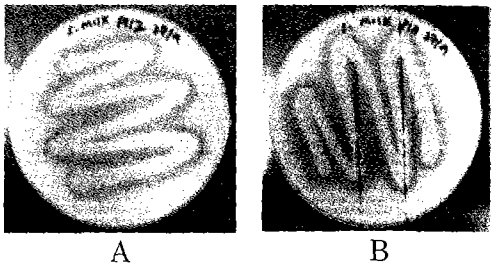


Fig. 8.: Hydrolisis zone surrounding the PI A (photo A) and PI 12 (photo B) colonies on skim milk agar plate after 7 days incubation at 5°C.

3.3 Cloning and characterisation of glycolitic genes

The full length of interest genes were obtained from the genome walking approach.

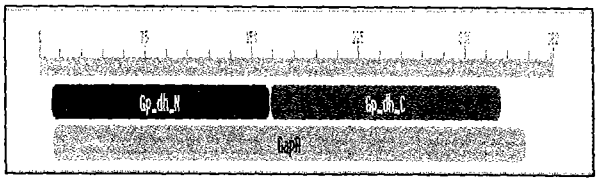


Fig. 9: ORF analysis of GAPDH gene.

The Blast-X result for cDNA of GAPDH gene revealed 72% identity to GAPDH of *Cryptococcus neoformans* JEC21. While for TPI, the Blast-X result for genomic DNA showed 53% identity to *Paracoccidioides brasiliensis*.

3.4 Purification of ATP-PFK

Purification Step	Vol. (mL)	Protein (mg)	Activity (mU)	Specific activity (mU/mg)	Yield (%)	Purification (fold)
Cell-free extract	52	55.12	252	4.56	100	1
Desalting (Sephadex G-25) & Ultrafiltration	38	32.0	153	4.78	61	1.05
Affin. Chrom. (Blue-Sepharose)	20	13.0	72.4	5.57	29	1.22
Anion Exchange (Q-Sepharose)	6	2.85	16.9	5.92	7	1.30

Table 2: Purification of the ATP-PFK from PI 12

The ATP-PFK purification yielded 2.85 mg enzyme at a purification fold of 1.3 with specific activity of 5.92mU/mg and recovery of 7%. The purified PFK was homogenous on SDS polyacrylamide gel with an estimated visual inspection molecular mass of 100 kDa.

4. Conclusion

In conclusion, the psychrophilic Antarctic yeasts were successfully isolated and identified. The production of potential industrial enzymes and metabolic enzymes from the microorganisms were identified for further studies to be carried out.

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