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CLONING OF LIPASE GENE FROM PSYCHROPHILIC MICROORGANISM ISOLATED FROM ANTARCTICA FRESH WATER SAMPLE

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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 and, in some cases, unusual specificity.

An obligately psychrophilic microorganism, which hydrolyses lipids at 5°C, was isolated from fresh water sample at Davis Station, Antarctic. The isolate is a rod-shaped, gram-positive bacterium with budding and size around 4.2µm. The isolate grows at 5°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 (Kouker & Jagger, 1987) 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 1.647U/ml. The genomic DNA of PI A was successfully extracted by using phenol-chloroform extraction with the modification of CTAB (cetyltrimethylammonium bromide) method from plant. 23kb of genomic DNA size was obtained. The extracted genomic DNA from PI A will be used for cloning and expression of lipase gene works. Isolation of lipase gene using PCR method was done using primers designed based on oxyanion hole and active site of selected lipases. The PCR product of these primers was obtained about 350bp. The PCR product was clone using pGEM®-T Vector System and *E. coli* JM109 as host. Plasmids were extracted and were sent for sequencing. The PCR product suspected to be α/β hydrolases. To confirm that this gene fragment was a functional lipase gene, further analysis is being carried out.