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Extracellular lipases from anaerobic microorganisms of Antarctic

Pramod W Ramteke*, Babu Joseph and M Kuddus

Department of Biotechnology, Allahabad Agricultural Institute – Deemed University, Allahabad 211 007, India

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Anaerobic microorganisms of Antarctic were investigated for production of extracellular lipases. Of 137 anaerobic strains studied, 49 (35.7%) strains showed lipolytic activity. Amongst the strains studied, 64 were psychrophiles (29 strict, 35 facultative) and 73 psychrotrophs (31 strict, 42 facultative). Of lipase producing psychrophiles, 9 (31.0%) were strict and 11 (31.4%) facultative anaerobes, whereas amongst lipase producing psychrotrophs, 13 (41.9%) were strict and 16 (38.0%) facultative anaerobes.

Keywords: Antarctica, anaerobe, lipase

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Lipases (triacylglecerol acylhydrolases, EC: 3:1.1.3) are hydrolases acting on the carboxyl ester bonds present in acylglycerols to liberate organic acids and glycerol. Many microorganisms such as bacteria, yeast and fungi are known to secrete lipases during growth on hydrophobic substrates. Lipases have generated considerable interest due to their industrial applications in detergent industries, flavour development in food processing industries, processing of fats and oils, application in organic synthesis, production of optically active compounds, polymer synthesis, and intramolecular esterification[1,2].

Numerous organisms, particularly bacteria, yeast, unicellular algae and fungi have been found successfully colonizing cold environments, the most abundant environment on the surface of our planet[3]. Despite extremely harsh climatic conditions, Antarctica continent is also enriched and dominated by a variety of microorganisms[4-6], which could be potential source of cold adapted enzymes. When a target enzyme type, one with commercial potential, has been established, extensive screening of bacterial isolates can be initiated. Examples may include lipases and proteases as they have the broadest applicability[7]. Lipases produced by psychrophilic Antarctic bacteria have been investigated[8,9]. The Indian effort to understand the Antarctic microbiology is limited to isolation and characterization of the microbes from the maritime Antarctic water, soil and sediments from the area of old Indian station "Dakshin Gangotri"[10,11]. However, to the best of our knowledge, lipases isolated from Antarctica anaerobes are not reported in India. Therefore, anaerobes obtained from Antarctica were screened for extracellular lipase production.

Thirty-two soil samples were collected from the vicinity of Indian station "Maitri" of the Schirmacher Oasis (Queen Maud Land/Dakshin Gangotri hill ranges), 70 km away from Princess Astrid coast of Antarctica between the inland and shelf ice occupying an area of 35 Km^2, situated between the geographical coordinates 70º45'12" to 70º46'30" S latitude and 11º22'44" to 11º54'00" E longitude. During the sampling period (January to February 1999), the average soil temperature was in the range of –3 to 3ºC. Most of the soil samples were collected under the mat of mosses and edges of lakes. For collection of soil samples, about 22-30 cm of surface soil was cleared with sterile spatula, and the underlying soil was collected and transferred to sterile sealed polythene bags.

Viable anaerobes were obtained by following standard microbiological methods using anaerobic agar (BBL, India) media (*p*H 7.0 ± 0.2). All the soil samples were plated in triplicate after appropriate dilutions to determine anaerobic population both at room temperature 26±2ºC as well as at freezing temperature (4ºC) and were incubated in anaerobic jars (E. Merck).

Lipase activity was detected by Tween 80-agar method[12]. Nutrient agar medium (BBL India) was supplemented with $CaCl_2.2H_2O$, 0.01%. Tween 80, sterilized for 20 min at 120ºC was added to the molten agar medium at 45ºC to give a final concentration of 1%. The medium was shaken until the Tween 80 had dissolved completely and then the isolates were streaked on to the petriplates. The plates were incubated at 4ºC for 3-7 days under anaerobic condition. An opaque halo zone occurring around the colonies indicated the positive test.

*Author for correspondence:
Tel: 91-532-2684296. Fax: 91-532-2684593/2684394
E-mail: pwramteke@yahoo.com

Table 1—Extracellular lipase from Antarctic anaerobic microorganisms

Organisms tested	No. of organisms	No. of organisms showing lipolytic activity
Psychrophiles		
Strict anaerobes	29	9 (31.0%)
Facultative anaerobes	35	11 (31.4%)
Psychrotrophs		
Strict anaerobes	31	13 (41.9%)
Facultative anaerobes	42	16 (38.0%)
Total	137	49 (35.7)

Anaerobes, comprising both psychrophiles and psychrotrophs, isolated from Antarctica were included in the study. Of 137 anaerobes studied, 49 (35.7%) strains showed lipolytic activity (Table 1). Amongst lipase producing psychrophiles, 9 (31.0%) were strict and 11 (31.4%) facultative anaerobes. Similarly, amongst lipase producing psychrotrophs, 13 (41.9%) were strict and 16 (38.0%) facultative anaerobes. Thus, of both psychrophilic (64) and psychrotrophic (73) anaerobes, more facultative anaerobes showed lipase activity as compared to strict anaerobes. Further, the results suggest that a large proportion of cold adapted anaerobes from Antarctica secrete lipases. Cold adapted lipases have various industrial applications. The largest use of lipases is in detergent industry. In cold washing, reduction in energy consumption and less wear and tear are obvious advantages[13] of such lipases.

Lipases are involved in the recycling of insoluble organic material in nature[13]. The recycling of material leads to what has been called clean technology, in which materials are systematically used and reused to bring about drastic increase in resource productivity, needed to make human activity sustainable[14]. In temperate regions, large seasonal variations in temperature reduce the efficiency of microorganisms in degrading pollutants, such as oil and lipids. However, the enzymes of cold adapted microorganisms, as a result of high catalytic efficiency and unique specificity at low and moderate temperatures, may also be ideal for bioremediation purposes. Although, the studies on taxonomy, ecology, biochemistry and physiology of psychrophilic organisms have only recently been started but the cold adapted microorganisms have much more in store to be exploited in future.

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