



SCREENING AND IDENTIFICATION OF LIPASE PRODUCING BACTERIA FROM DOMESTIC WASTEWATER

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ABSTRACT

The ability of lipases is to perform very specific chemical transformation and it has made them increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries. The domestic wastewater is one of the good sources for isolation of lipase producing microorganism. Total 40 isolates were isolated from waste water on nutrient agar medium. All were screened for lipase activity on Tributyrin agar (TBA). Out of these, 10 bacterial isolates showed lipase activity. Out of these four isolates were found as maximum lipase producer. The isolate showing maximum activity was identified by following Bergey's Manual of Determinative Bacteriology. They were belongs from genera *Bacillus* sp. and *Panibacillus* sp.

KEYWORDS: Lipase, *Bacillus* sp., *Panibacillus* sp., Domestic wastewater



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INTRODUCTION

Lipase (triacylglycerol acylhydrolases, EC 3.1.1.3), a large series of enzymes that catalyze the hydrolysis of triacylglycerols into diacylglycerols, monoacylglycerols, fatty acids and glycerol. The lipase from microorganisms are most interesting because they are often more useful than lipase derived from plants or animals because of the great variety of catalytic activities available, the high yields possible, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations, and rapid growth of microorganisms on inexpensive media¹. Lipases are a subclass of esterase widely spread in animals, plants and microorganisms². Among them, microbial enzymes are often more stable, economical and safer³. Lipolytic enzymes are highly diversified in their industrial application. They have emerged as key enzymes which find usage in food, dairy, paper, textile, leather and detergent industries, waste water treatment, production of fine chemicals, pharmaceuticals and cosmetics, synthesis of surfactants and polymers, vegetable fermentation and meat product curing. Lipase producing bacteria have been found in diverse habitats such as soil contaminated with oil, dairy waste, industrial wastes, oil seeds and decaying food, compost heaps, coal tips and hot springs⁴. The aim of present study was to isolate lipase producing bacteria from the domestic wastewater.

MATERIALS AND METHODS

Sample collection

For the present study, domestic wastewater samples were collected from various places from Buldana District (M.S.). Samples were aseptically transferred to the laboratory of Shri Shivaji Science College Chikhli Dist. Buldana (M.S.) for further analysis.

Isolation of lipase producing bacteria

Samples were serially diluted with sterile distilled water and spread on the nutrient agar plates by spread plate method followed by incubation for 24-48 h at 37 °C for the growth of bacteria. Microbial colonies, which appeared on nutrient agar plates, were purified. All isolates were subjected for screening of lipase production on tributyrin agar plates at 37°C for 24, 48 and 72 hrs. The zone of clearance were observed around the colonies indicated the lipolytic activity of that isolates. Similarly all bacterial isolates were preserved at 4^oC for further study⁵.

Identification of bacterial isolates:

Bacterial isolates were examined for colony characteristics and cell morphology. Biochemical tests were also performed such as Catalase, Oxidase, IMViC test, H₂S, Sugar fermentation etc. The isolated bacteria were identified according to Bergey's Manual of Determinative Bacteriology.

RESULTS AND DISCUSSION

Total 40 bacterial isolates were obtained on nutrient agar medium on the basis of morphological characters. All isolates were studied for lipase activity on TBA plates for 24, 48, 72 hr at 37^oC. Out of this, only 10 bacterial isolates were showed lipase activity. Those isolates which showed more than 10 mm zone of clearance after 24 hr were used for further study. Four bacterial isolates were showed more than 10 mm zone of clearance after 24 hr. On the basis of Bergey's Manual of Determinative Bacteriology, these isolates were identified as *B. megaterium*, *B. acidiceler*, *B. Cereus*, *P. borealis* and *P. amylolyticus*.

Table 1
Bacterial isolates showing zone of clearance at different time

Isolates	Zone of clearance in mm		
	24 hr	48 hr	72 hr
<i>B. megaterium</i>	10	21	36
<i>B. acidiceler</i>	14	24	41
<i>B. cereus</i>	25	62	80
<i>P. borealis</i>	12	26	38
<i>P. amylolyticus</i>	10	17.5	34

Each bacterial isolates mentioned above showed increasing enzyme activity as time increases. *B. cereus* was showed maximum enzyme activity as compared to other bacterial isolates whereas *P. amylolyticus* showed minimum lipase activity. From the table no.1, at 24 hr zone of clearance were 10, 14, 25, 12, 10 mm of *B. megaterium*, *B. acidiceler*, *B. cereus* *P. borealis* and *P. amylolyticus*, respectively. *B. cereus* showed maximum zone of clearance at 24 hr while *B. megaterium* and *P. amylolyticus* showed minimum zone of clearance. At 48 hr zone of clearance were 21, 24, 62, 26, 17.5 mm for *B. megaterium*, *B. acidiceler*, *B. cereus* *P. borealis* and *P. amylolyticus*, respectively. *B. cereus* showed maximum zone of clearance at 48 hr while *P. amylolyticus* showed minimum zone of clearance. At 72 hr zone of clearance were 36, 41 80, 38, 34 mm of *B. megaterium*, *B. acidiceler*, *B. cereus* *P. borealis* and *P. amylolyticus*, respectively. *B. cereus* showed maximum zone of clearance at 72 hr, while *P. amylolyticus* showed minimum zone of clearance. Similar result was obtained by Sanghamitra Dutta & Lalitagauri Ray in 2009 while studying Production and

Characterization of an Alkaline Thermostable Crude Lipase from an Isolated Strain of *Bacillus cereus* C7. Kim BC *et al.*, in 2009 also studied *Paenibacillus pini* sp. nov., a cellulolytic bacterium isolated from rhizosphere of pine tree. Similar result also found by Teeraphatpornchai *et al.*, in 2003 while studying Isolation and characterization of a bacterium that degrades various polyester-based biodegradable plastics.

CONCLUSION

The domestic wastewater is one of the good sources for isolation of lipase producing microorganism because it contains lipid material from diet and other household activities. So there is major possibility to get robust bacterial isolates, which will help for more and more lipase production. In this study ,we got four bacterial isolates which showed maximum lipase activity and they were belonging from *Bacillus* sp. and *Paenibacillus* sp. on the basis of Bergey's Manual of Determinative Bacteriology.

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