World Applied Sciences Journal 12 (10): 1798-1802, 2011
ISSN 1818-4952

Production of Lipase Enzyme from *Lactobacillus* spp. and Its Application in the Degradation of Meat

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Abstract: Bacteria isolated from various samples were screened for lipolytic activity. Optimization of fermentation conditions such as temperature and pH for maximum lipase production was examined. From the results obtained, it was showed that *Lactobacillus* sp has more lipase activity when compared to *Bacillus* sp and *Pseudomonas* sp. Partial purification of crude enzyme produced from *Lactobacillus* sp was carried out by ammonium sulphate precipitation and dialysis. The lipase was active at pH 9 and 40°C and was found to be stable between pH 8 - 9 and 30 - 50°C. The activity of lipase on degradation of meat was also studied to apply them in industry.

Key words: Bacterial lipase · *Bacillus* sp · *Lactobacillus* sp · *Pseudomonas* sp · Optimization · characterization

INTRODUCTION

The demand for industrial enzymes, particularly of microbial origin, is ever increasing owing to their applications in a wide variety of processes. Lipases, triacylglycerol hydrolases, are an important group of biotechnologically relevant enzymes and they find immense applications in food, dairy, detergent and pharmaceutical industries [1]. Lipase enzymes are active at the interface of aqueous and non-aqueous phases which distinguishes them from esterase's [2]. The interest in microbial lipase production has increased in the last decades, because of its large potential in industrial applications [3, 4]. Lipases are available from many sources however, the most suitable sources for lipase production are microbes including bacteria, fungi and yeast. These microorganisms can produce high quality lipases in lower cost and shorter time [5]. In order to get the highest yields of lipase, the optimal growth conditions should then be considered [6]. Lipase production is dependent upon a number of factors including carbon and nitrogen sources, pH, temperature, aeration and inoculum size [7, 8].

Lipases are widely used in the processing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, paper manufacture and production of cosmetics and pharmaceuticals [9-11]. Lipase can be used to accelerate the degradation of fatty waste [12] and polyurethane [13]. The purpose of the present study was to screen lipase producing organisms using Tributyrin agar. 47 Bacterial strains were isolated from various samples (soil, coconut, milk). Among that, the genera *Pseudomonas, Bacillus* and *Lactobacillus sp,* grew on the Tributyrin agar and produced lipolytic activity. Partial purification was obtained by ammonium sulphate precipitation and dialysis. Production of lipase was optimized and characterized [14].

MATERIALS AND METHODS

Collection of Samples: Samples such as soil, coconut, milk were collected from different places of Calicut and Aluwaye were subjected to serial dilution. Serially diluted samples were plated to nutrient agar plates and kept for incubation at 37°C for 24-48 hours and the plates were observed for growth.

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Isolation and Identification of Bacteria: Isolated bacterial cultures were examined for various morphological and biochemical characteristics as per Bergey's Manual of determinative Bacteriology. The bacterial strains were deposited at Karpagam Microbial Culture Collection Centre (KMCCC), Coimbatore, India.

Lipolytic Activity: For preliminary screening of lipase producing bacteria, tributyrin agar was used. All the isolated cultures were inoculated into tributyrin agar plates and kept for incubation at 37°C for 24 hours and observed for zone formation [15]. A clear zone around the colonies indicates the production of lipase.

Fermentation [16]: Fermentation was carried out in shake flask using a complex medium consisting of (g/l) olive oil, 20 (emulsified in gum acacia), egg yolk, 10; ammonium chloride, 4.0; magnesium sulphate heptahydrate, 0.25; dipotassium phosphate, 0.5; calcium carbonate, 0.5. The flask containing 40mL fermentation medium were inoculated by 18 h old vegetative inoculum. The vegetative inoculum was developed from spore suspension prepared from 48 h old culture slants. The inoculum development and the fermentations were carried out at 30°C for 7 days at 100 rpm and the calorimetric reading was taken.

Optimization [17]: Lipase production was optimized at different pH (7 - 10), temperature (30 - 60°C) and incubation period (1-5 days) with constant shaking at 120 rpm. Bacteria were cultured in nutrient broth with 1% olive oil. During the cultivation, lipase activity will be measured every 12 h to determine the maximum lipase producing period.

Partial Purification of Lipase: The culture supernatant containing extracellular lipase obtained from fermented broth was treated with 0.4M CaCl2 in order to precipitate fatty acids followed by centrifugation at 4°C and 12,857g for 30 min. The supernatant was collected in a glass beaker and to it chilled acetone was added slowly, with continuous stirring, up to 70% (v/v) concentration and kept at 20°C for 4 h to allow protein precipitation. The precipitates were then harvested by centrifugation at 4°C and 12,857g for 30 min. The pellet thus obtained was resuspended in 34 mL of 20 mM Tris-HCl buffer (pH 8.0) to allow the solubilization of proteins. The unsolubilized proteins were then removed by centrifugation at 4°C, 12,857g for 30 min. Supernatant was then subjected to ultrafiltration and dialyzed overnight against same buffer at 4°C. The protein content and lipase activity were determined after each step.

Enzyme Characterization

Determination of Optima and Stability of Temperature and pH: Optimum temperature for activity of the lipase was determined by carrying out at selected temperatures from 30 to 70°C. In each case, the substrate was preincubated at the required temperature before the addition of enzyme. The optimum pH was determined by monitoring lipase activity at pH values between 6- 10.

Application Study: Lipases are endowed with a substrate specificity that surpasses that of any other known enzyme. This confers on these enzymes an application potential that is literally boundless [18]. 5g of meat was weighed and it was autoclaved at 121°C for 15 lbs for 30 minutes. The enzyme filtrate was added to meat and incubated at 37°C for 72 hours for the observation of the lipolytic activity on the meat.

RESULT AND DISCUSSION

Samples Collected: During isolation, 47 isolates were obtained which was then inoculated to tributyrin media and observed for zone formation. Among the 47 isolates 3 isolates (KMCCCB301, 302, 303) were found to produce maximum zone formation of 15, 20 and 35 mm, respectively.

Microbiological Examination: Based on microbiological and biochemical identification of the three isolates (Table 1) it was identified as *Pseudomonas* sp (KMCCCB301), *Bacillus* sp (KMCCCB302) and *Lactobacillus* sp (KMCCCB303).

Fermentation: After fermentation, when turbidity was checked using spectrophotometer, *Lactobacillus sp* showed absorbance of 1.34 which was higher when compared to *Bacillus sp* (1.15) and *Pseudomonas sp* (1.20).

Enzyme Assay: From Table 2, the enzyme activity was observed higher in case of *Lactobacillus* sp (35 U/ml) when compared to *Bacillus* sp and *Pseudomonas* sp. So *Lactobacillus* sp (KMCCCB303) was used for further study.

Table 1: Microbiological and biochemical examinations[1] for three isolates which produced maximum zone formation

Sl No.	Organisn	Gram staining	Motility	Indole test	MR test	VP test	Cirate test	Gelatin test	Urease test	Catalase test	Sugar Fermentation G	L	S	M
1	KMCC CB301	Gram negative rod	Motile	-	-	+	+	+	+	+	-/-	-/-	-/-	-/-
2	KMCC CB30	Gram positive rod	Motile	-	-	+	+	+	-	+	-/-	-/-	-/-	-/-
3	KMCC CB 30	Gram positive rod	Non motile	-	-	-	-	+	-	-	A/-	A/-	A/-	A/-

Table: 2 Lipase activity of the selected isolates

Sl No.	Organism	Lipase activity (U/mL)
1	Pseudomonas sp (KMCCCB301)	10
2	Bacillus sp (KMCCCB302)	18
3	Lactobacillus sp (KMCCCB303)	35

Table 3: Partial purification of lipase enzyme

Fractions	Totalvolume (ml)	Enzyme Activity (U/ml)	Protein (mg/ml)	Total protein (mg)	Total Activity (U/ml)	Specific activity (U/ml)	Purification (Fold)	Recovery (%)
Culture Filtrate	100	16	6.2	1329	1600	2.5	1.00	100.0
Ammonium sulphate precipitation	50	24	4.6	462	1200	5.21	2.08	75.0
Dialysis	25	32	3.5	187	800	9.14	36.50	66.6

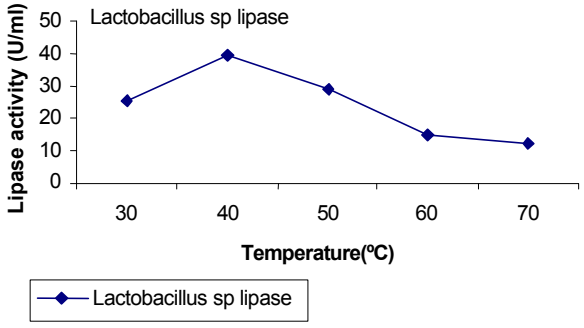

Fig. 1: Lipase production at different temperature

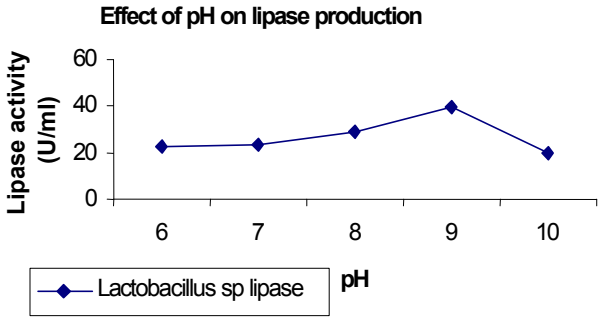

Fig. 2: Lipase production at different pH

Total Protein Estimation by Lowry's Method: The total extracellular protein obtained from the culture filtrate was partially purified by ammonium sulphate precipitation and was estimated by Lowery's method [19]. The total protein content of *Lactobacillus sp, Bacillus sp* and *Pseudomonas sp* was found to be 1.8mg/ml, 1.63mg/ml and 1.2mg/ml respectively.

Lee *et al.* [20] showed specific activity of 7395 U/mg protein for alkaline lipase (pH 8.5) produced by *P. fluorescens* S1K WI in a medium which contained emulsified olive oil as the carbon source.

Optimization Studies: Lipase production at different temperature (30 - 70°C) and pH (6 - 10) were examined. Lipase production increased with increase in temperature from 30 to 40°C. Maximum production of lipase (39 U/ml) was obtained at 40°C and production declined at 50°C. Although many other enzymes produced by bacteria and yeast show maximum activities at high temperatures, such as *Pseudomonas aeruginosa* (70°C) (Karadzic *et al*., 2006) and yeast *Kurtzmanomyces* sp. Lipase production by *Lactobacillus sp* was observed on different pH range from 6-10. Growth and lipase production ceased at pH 10. Maximum lipase production of 39.6 U/ml was observed at pH 9.

Partial Purification of Lipase Enzyme: The purification of lipase resulted in 2 fold purification with 75% recovery by ammonium sulfate precipitation. The purification of crude enzyme through dialysis gave purification fold of 36.5 with 66.6% recovery of lipase from *Lactobacillus sp*. (Table 3).

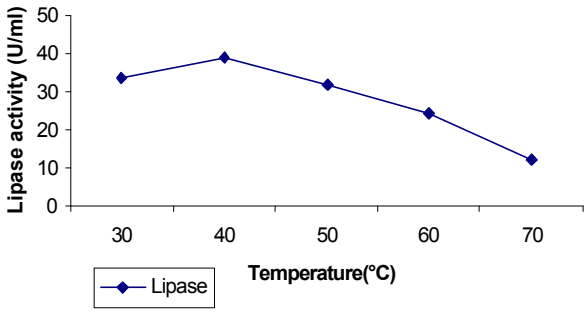

Fig 3: Effect of various temperature on lipolytic activity

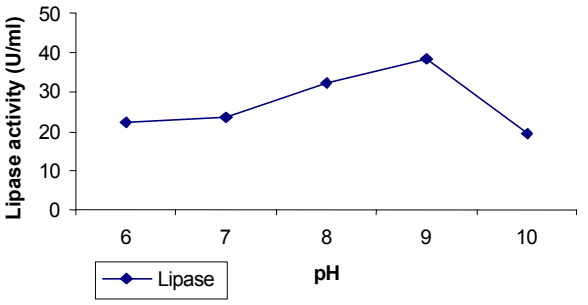

Fig. 4: Effect of various pH on lipolytic activity

Characterization Study: The residual activity was measured to determine lipase stability after one hour incubation at different temperature ranging from 30-70°C. The maximum stability of the enzyme was observed in the tempertature range of 30 to 50°C, when the enzyme was incubated for 1 h (Fig. 3). So the thermo stability of the enzyme was found to be up to 50°C for *Lactobacillus* sp. The effect of pH on the activity of lipase was studied with various pH from 6-10. The optimum pH for lipase enzyme from *Aspergillus flavus* was determined as 9 (Fig. 4). The pH stability curve showed that the lipase was stable in the range of pH 8 -9. Most microbial lipases are stable in the pH range 2 to 10.5 as reported by many researchers. Similar results have been reported for other microbial lipases [21].

Application Study: After the incubation period, the beginning of degradation of meat was observed along with a very strong smell. This may be because of the lipid present in the meat get degraded by lipase enzyme which can be applied in medical field in removing excess fat content.

The present work can be summarized as the production of lipolytic enzymes by *Bacillus* sp, *Lactobacillus* sp, *Pseudomonas* sp by fermentation. The maximum lipase activity reached was 32 U/ml with a purification fold of 36.5 and a yield of 66.6 %. It was concluded that *Lactobacillus* sp could be used as a new potent microbial source of lipase. The activity of lipase on meat was also studied to apply them in industry. Further studies are recommended on the use of various other bacteria and other strains of *Bacillus* sp, *Pseudomonas* sp and *Lactobacillus* sp for much better lipolytic activity.

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