

Production of extra cellular lipase from *Bacillus* sp LBN 4 by solid state fermentation

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Abstract

Bacillus sp LBN4 produced lipase during solid substrate fermentation. Maximum lipase production was 14.0IUg⁻¹ with rice bran as sold substrate. 50% more lipase production was achieved when rice bran was supplemented with soyabean mixture. Supplementation of the medium with wheat bran led to 70% increase in lipase production. Optimum temperature and pH were 50 °C and 7.0 respectively. Na⁺ induced more lipase than K⁺ and Mg⁺⁺

INTRODUCTION

Lipases are hydrolytic enzymes that catalyses the cleavage of ester bonds in triglycerides and producing glycerol and free fatty acids. These are biotechnologically relevant enzymes and find potential applications in detergent, food, pharmaceutical, leather, paper and pulp industries₁. However for commercial applications development of low cost processes and production of lipases using simple and inexpensive substrates such as agro industrial residues are more favorable. Solid state fermentation is a low cost alternative and involves the growth and metabolism of microorganisms on moist solids without free flowing water. Solid state fermentation (SSF) has many advantages over submerged fermentation (SmF) including simplification of the fermentation media, low capital investment, absence of complex machinery, reduced energy requirement and improved product recovery (Losane et al 1983; Satyanarayana 1994). Major group of microorganisms used in Solid state fermentation are bacteria, actinomycetes, fungi and yeast. The vast majority of literature on Solid state fermentation refers to the fungi and yeast using different solid substrates (Pandey et al 1999; Rao et al 1993; Falony et al 2006; Kamini et al 1997) The exploitation of new microorganisms capable to produce lipase in Solid state fermentation conditions would be useful for industrial applications in the near future. A strain of *Bacillus* Sp LBN 4 was taken from stock repository which was originally isolated from the alkaline soil of hot spring of Arunachal Pradesh was investigated for production of extra cellular lipase by solid state fermentation using rice bran as a solid

substrate.

Solid state fermentation was carried out in 250ml conical flasks. 10 g of rice bran was mixed with mineral salt medium and final moisture ratio of 1:2 w/v was achieved. The pH of the mixture was adjusted to 7.0 in sodium phosphate buffer. The above mixture was autoclaved at 121 °C for 20min, cooled to room temperature and inoculated with 10% of 12h grown seed culture of *Bacillus* sp and incubated at 40 °C for different time intervals (12, 24, 36,48h). Enzyme extraction was carried out by adding 10 ml of 0.2M phosphate buffer having pH 7.0 to each flask and agitating in the orbital shaker at 200rpm for 1h. The particulate matter was filtered through a muslin cloth and centrifuged at 10,000rpm for 30 minutes. The clear supernatant was used for lipase assay.

In another experiment, effects of various cheap substrates like soyabean oil, soybean meal, Wheat bran and coconut oil was studied by adding each of the substrate in the rice bran mixture and the lipase activity was determined accordingly.

Lipase activity was determined by (Watnabe et al method 1977). The reaction mixture containing 5ml of olive oil emulsion composed of 25 ml olive oil and 75 ml 2% polyvinyl alcohol solution, 4ml of 0.2M tris buffer, 1ml of 110mM CaCl₂ and 1ml enzyme solution. The control containing boiled inactivated enzyme (at 100 °C for 5 minutes) was treated similarly. After the incubation, the enzyme activity was blocked by 20 ml of acetone ethanol (1:1) mixture and liberated free fatty acid was titrated against 0.02 M NaOH using phenolphthalein as indicator. One unit

of lipase was defined as the amount of enzyme, which liberates 1 μ mol of fatty acid/ min under standard assay conditions. The enzyme activity was expressed as Ug^{-1} dry substrate.

The effect of pH on lipase production was studied in a pH range of 5- 10.0 using different buffers (citrate buffer 3-6, sodium phosphate, 6-7, Tris HCL pH8.0 and glycine NaOH 9-10.0) at 50mM concentration. Temperature effect on lipase production was determined by carrying out the incubation at different temperatures in the range of 30-80 ° C at pH 7.0. The effects of metal ions were also studied.

The maximum lipase yield from *Bacillus* sp LBN 4 with rice bran as a substrate was found to be (14.0IUg⁻¹ wet weight) obtained after 36 hours of incubation. The enzyme activity was found to be decreasing on further incubation. The addition of soybean oil results in the marginal increase in the lipase yield (10%) whereas soybean mixture enhanced maximum (50%) of the lipase activity when used as a substrate. (Table 1)

Table 1: Effect of different substrates on lipase production by *Bacillus* sp in solid state fermentation

Figure 1

Substrates	Lipase activity (IUg ⁻¹)			
	12	24	36	48
Rice Bran	8.0	12.6	14.0	10.8
Rice Bran+ Soyabean oil	7.6	14.0	16.0	12.6
Rice Bran+ Soyabean Meal	10.6	18.6	21.0	20.8
Rice Bran+ Wheat Bran	8.6	14.0	23.5	14.8
Rice bran + Coconut oil	7.2	12.8	18.0	16.8

The increase in lipase production may be due to the presence of additional factors like nitrogen and oil compounds which acts as stimulating factors in the medium. The addition of wheat bran increased the yield upto 70% whereas marginal increase was obtained with coconut oil (20%). Sekhon et al₁₀ reported 42% of increase in the lipase yield from *Bacillus megaterium* AKG 1 using wheat bran as a soild substrate. 17% increase in the lipase yield has been reported in *Candida rugosa* grown on rice bran as soild substrate (Rao et al 1993). Increased lipase production from alkalophilic yeast was observed when Rice bran and wheat bran were used as substrate (Bhushan et al 1994) 4.8 IU/ml of lipase activity was found in *Aspergillus niger* when wheat bran was used as substrate (Faloni et al 2006) . Babassu oil cake was

used for lipase production by *Penicillium restrictum* (Gombart et al 1999) whereas wheat rawa supplemented with corn steep liquor and olive oil was studied by (Adinarayana et al 2004).

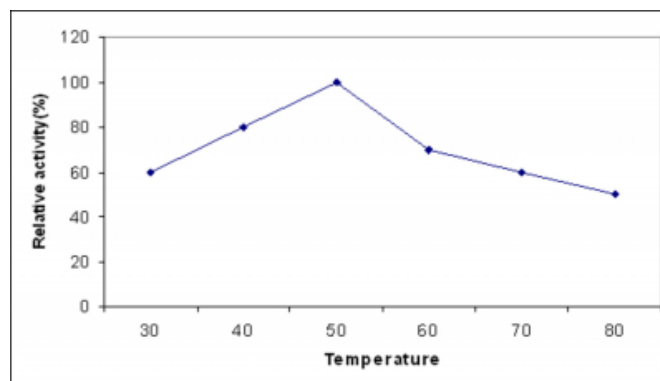
In most of the earlier reports the lipase production was reported after prolonged fermentation period (72-192h) whereas in our study the maximum lipase yield was observed after 36h, (Sekhon et al 2004) reported maximum lipase production after 48h..

Lipase from *Bacillus* sp showed optimal activity at 50 °C (Fig 1) . The optimum temperature for lipase production corresponds with the given temperature of the respective microorganism. The best temperature for growth of lipase production in case of *Bacillus* sp RSJ1 was found to be 50 ° C

(Sharma et al 2002) . Similar results were also observed in *Bacillus* sp (Khayami H 1996).

Figure 2

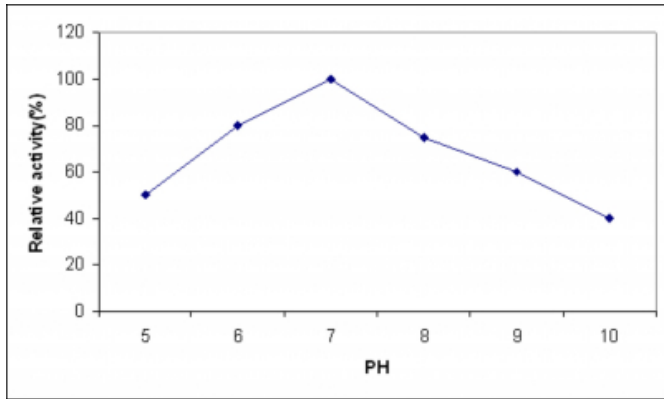
Figure 1: Effect of incubation temperature on lipase production.



The optimum lipase activity was observed at pH 7.0 (Fig 2). The activity was found to be decreasing on increasing the pH values. The initial pH of the growth medium was important for lipase production. The pH in and around 7.0 was found to be preferable for bacteria for better growth and lipase production. Similar results have been reported for other *Bacillus* lipases (Sugihara et al 1991).

Figure 3

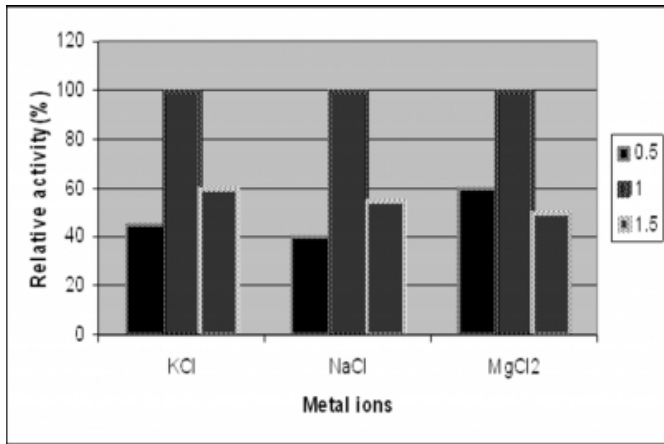
Figure 2: Effect of pH on lipase production.



Divalent cation plays an important role in enzyme production. Earlier reports suggested that K^+ and Mg^{++} are essential for lipase production. To study the effect of metal ions KCl, NaCl and $MgCl_2$ at a concentration of 0.5, 1 and 1.5% (w/v) were added to the medium and incubated. Optimum concentration was found to be 1% in all three cases (Fig 3). The best lipase production was achieved with Na^+ followed by K^+ and Mg^{++} . Similar results of lipase stimulation have been reported (Van ort et al 1989).

Figure 4

Figure 3: Effect of Metal ions on lipase production



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