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Immobilization and kinetic study of lipase enzymes from *Lactobacillus plantarum* and *Lactobacillus brevis*

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Abstract: Enzymes are biological catalysts which are used in promoting the rate of reactions. The repeated use of enzyme in different solvents results in loss of enzyme activity and cannot be economically recovered. It is important to choose an enzyme which has fastest reaction rate, efficiency and stability for industrial scale production. Hence immobilization and enzyme kinetics play an important role in enzymology. In the present study lipase from *Lactobacillus brevis* and *Lactobacillus plantarum* were immobilized in sodium alginate beads and kinetic study was done using p-nitrophenyl palmitate as substrate. Upon immobilization the enzyme showed optimal conditions of pH7 and 40°C temperature for Lactobacillus brevis and pH 65 and 45°C temperature for Lactobacillus plantarum. In terms of stability and efficiency, the immobilization of lipase by sodium alginate beads showed good results over expressed enzyme and can be used in large scale production of industrial products.
Keywords: Lactic acid bacteria, Lipase, Immobilization, Sodium alginate beads, Kinetics.

1. Introduction

Enzymes are industrial biocatalysts that recognize the unique target molecule and undergo several biotransformation reactions [1]. Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) are one of the largest groups of such industrial catalysts which find use in different industries like detergents, pharmaceuticals, beverages, cosmetics, dairy, degreasing formulations, paper and biofuel [2]. They are also used as biosensors, diagnostic tool, in waste/sewage treatment and oil biodegradation [3-4].

The stability of enzymes can be improved by protein engineering, immobilization technique and chemical modification of enzymes [5] among which immobilization plays a vital role. The techniques used to immobilize is an important factor for large scale use in industry. Immobilization has several advantages like production of high purity products, easy separation of products and high pH and thermal stability [6]. Lipases are also immobilized on different supports with different immobilization techniques [7-10].

Lipase enzyme from *Pseudomonas aeruginosa* BBRC-10036 was immobilized on calcium alginate beads and optimized statistically by Response surface methodology. The immobilized enzyme showed an activity of 2.64 U/g [11]. Similarly a thermostable lipase enzyme produced from thermophilic *Bacillus sp.* was immobilized on silica and HP-20 beads by cross linking method that increased the thermostability of the enzyme [12]. Sodium alginate immobilized lipase from *Bacillus cereus* MS6 showed an increased activity of 550 U/ml than the activity of free enzyme [13].

The rate of activation and inactivation of enzymes and their functional efficiency together constitutes the kinetic data of an enzyme. The determination of K_m and V_{max} values of enzyme system is necessary to determine the kinetic potentiality of enzymes and their specificity. Lipase from *Pencillium chrysogenum* SNP5

was immobilized on silica gel and polyacrylamide beads and different kinetic properties like pH stability, K_m and V_{max} were studied. High thermostability, specificity and wide range of pH were observed after immobilization [14]. Acidic lipase derived from *Pseudomonas gesardii* was immobilized in Mesoporous activated carbon (MAC_{400}). The thermal stability and reusability of immobilized lipase was found to be better than native enzyme [15].

In the present study, the immobilization method and kinetics of lipase enzyme from probiotic lactic acid bacteria like *Lactobacillus brevis* and *Lactobacillus plantarum* has been reported along with its parametric behavior which was the first report using simple sodium alginate bead entrapment method.

2. Materials and Methods

2.1 Source of the enzyme

Probiotic lactic acid bacteria like *L. plantarum* and *L. brevis* (MTCC 4461 and MTCC 4460) were used as sources of lipase enzyme.

2.2 Preparation of sodium alginate gel (3%)

3g of sodium alginate was dissolved in 100ml of distilled water and mixed thoroughly to make a transparent gel and left undisturbed for 30 minutes to remove the air bubbles. After 30 minutes of incubation the gel was used for entrapment of the enzyme.

2.3 Determination of lipase activity

The lipase assay was performed spectrophotometrically using p-nitrophenylpalmitate as substrate. The assay mixture contained 2.5 ml of 420µm p-nitrophenylpalmitate, 2.5ml of 0.1 M Tris – HCl (pH-8.2) and 1ml of lipase solution. It was incubated in water bath at 37°C for 10 min. The p-nitrophenol was liberated from p-nitrophenylpalmitate by lipase mediated hydrolysis imparting a yellow color to the reaction mixture. After incubation, the absorbance was measured at 410 nm [16]. One unit (U) of lipase was defined as the amount of enzyme that liberates one micromole of p-nitro phenol per minute under the assay conditions [17-18].

2.4 Preparation of immobilized enzyme using sodium alginate

Sodium alginate gel was prepared by suspending 3g of sodium alginate in 100 ml distilled water [19], stirred for 5 minutes and incubated for 30 minutes. To this suspension 0.5ml of lipase enzyme was added and mixed thoroughly. Beads of uniform size were formed by dripping the solution in freshly prepared 250ml of chilled 0.2 M Calcium chloride. Beads were left in calcium chloride for 3-4 hours and then resuspended in Tris-Cl buffer (8.2). The beads were washed several times with the buffer to remove unbound enzyme. The enzyme activity of the beads was calculated. The units of enzyme entrapped in a gel were calculated by subtracting the units of non-entrapped enzyme from total units of the enzyme [20]. The total units of enzyme were 102 U/ml for *L. brevis* and 110 U/ml for *L. plantarum.*

2.5 Effect of pH on enzyme activity

To study the effect of pH on the immobilized lipase the enzyme was incubated in different buffers with pH 5-5.5 (acetate); pH 6-7 (phosphate) and pH 8-8.5 (Tris-HCl) at 4 °C for 24 h.

2.6 Effect of temperature on enzyme activity

A change in temperature may affect the stability of the enzyme leading to permanent loss of enzyme activity. Therefore to evaluate the effect of temperature on immobilized lipase, the enzyme was incubated at different temperatures ranging from 25 to 50 °C Tris–HCl buffer with pH 8.0 at an interval of 5 °C.

2.7 Effect of metal ions and inhibitors on enzyme activity

Effect of different metal ions like K^+, Na^+, Cu^{2+},$Fe^{2+}$and Mg^{2+} on the activity of immobilized enzyme was studied by incubating the enzyme with specified ion at 1mM concentration containing buffer (Tris–HCl with pH 8.0) and the activity was measured. Similarly the effect of different inhibitors like EDTA (ethylenediaminetetra acetic acid), SDS (sodium dodecyl sulphate) (at 1mM concentration), on enzyme activity was also studied.

2.8 Kinetic study

The Michaelis-Menten kinetic parameters V_{max} and K_m values of immobilized lipase were calculated using pNPP (p-nitrophenylpalmitate) as substrate at concentrations ranging from 0.5 mM to 2.5mM at an interval of 0.5mM.

3. Results and Discussion

3.1 Effect of pH

The immobilized lipase enzyme was optimally active at pH 7 for *L. brevis* and pH 6.5for *L. plantarum.* Further increase in pH caused rapid decrease in enzyme activity as shown in the Figure 1.

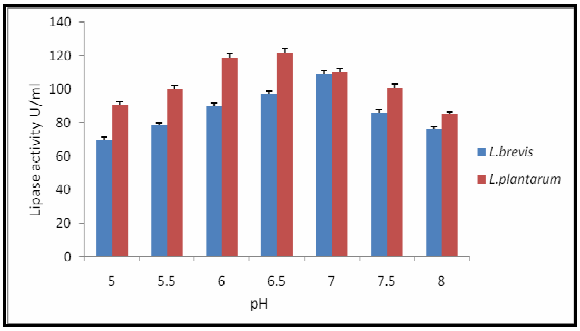

Figure 1 showing the effect of pH on immobilized lipase activity

3.2 Effect of temperature

The optimum activity of the immobilized enzyme was found to be 40°C for *L. brevis* and 45°C for *L. plantarum* as shown in Figure 2.

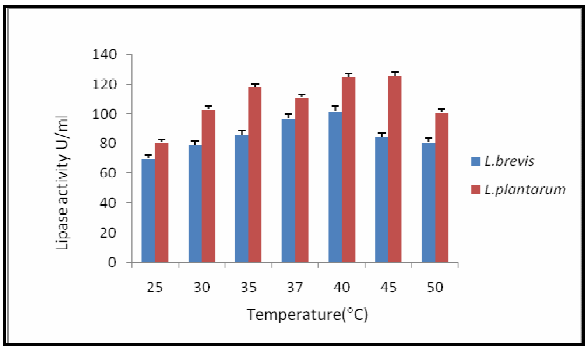

Figure 2 showing the effect of temperature on immobilized lipase activity

3.3 Effect of metal ions and inhibitors

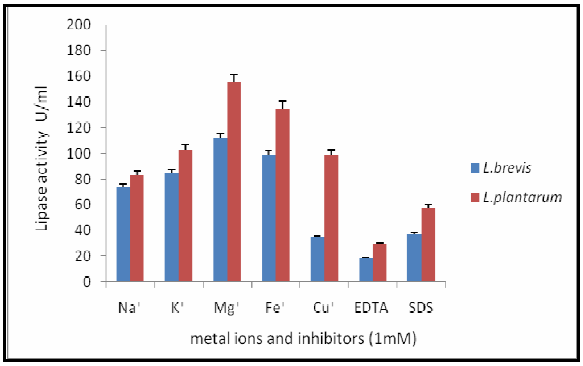

Figure 3 showing the effect of metal ions and inhibitors on the activity of immobilized lipase.

The effect of different metal ions and inhibitors on enzyme activity was studied and found that activity of immobilized enzyme was enhanced by Mg+ ions for *L. brevis* and *L. plantarum* where as EDTA and SDS decreased the activity of the enzyme as shown in Figure 3.

3.4 Kinetic study

The kinetic parameters V_{max} and K_m were determined from Lineweaver-Burk plots. The V_{max} and K_m values of the immobilized enzyme for *L. brevis* were 1.33µmol/mg/min and 0.39mM as shown in the Figure 4.

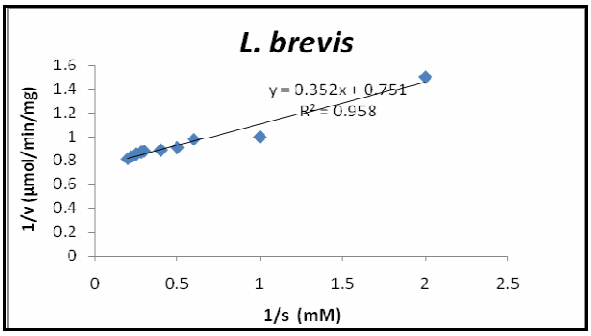

Figure 4 showing the V_{max} and K_m values of immobilized lipase of *L. brevis*

Similarly the V_{max} and K_m values of the lipase for *L. plantarum* were 1.47µmol/mg/min and 0.37mM respectively as shown in Figure 5.

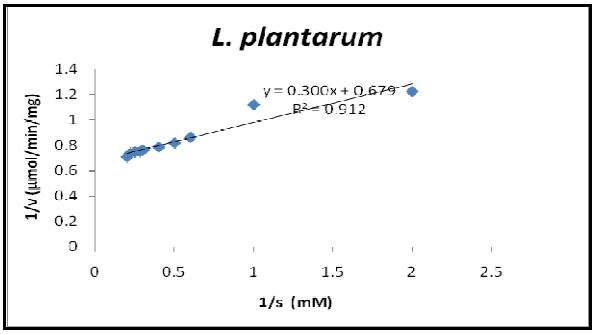

Figure 5 showing the V_{max} and K_m values of immobilized lipase of *L. plantarum*

The table given below shows a comparative study of the kinetic properties of the expressed and immobilized enzyme.

Table 1 showing the comparative study of kinetic properties of expressed and immobilized lipase from *L. brevis* and *L. plantarum*

Parameter	Expressed enzyme		Immobilized enzyme	
	L. brevis	***L. plantarum***	***L. brevis***	***L. plantarum***
pH	7	6	7	6.5
Temperature	37°C	40°C	40°C	45°C
Activators and Inhibitors	Mg^+ EDTA, SDS	Mg^+ EDTA, SDS	Mg^+ EDTA, SDS	Mg^+ EDTA, SDS
K_m	1.28µmol/mg/min	1.44µmol/mg/min	1.33µmol/mg/min	1.47µmol/mg/min
V_{max}	0.44mM	0.426mM	0.39mM	0.37mM

From the table 1 it was evident that there was shift in pH from expressed enzyme to immobilized enzyme of *L. plantarum* from pH 6 to 6.5. This might be due to change in H^+ ion concentration of the immobilized lipase in sodium alginate gel. But for *L. brevis* no change in pH was observed. Similarly temperature variation was also seen in expressed and immobilized enzyme which might be due to

hydrophilicity of alginate gel and hydrophobicity and flexibility of enzyme [21]. The K_m value of expressed enzyme was less than compared to immobilized enzyme which shows that enzyme substrate complex of expressed enzyme was more stable than that of immobilized enzyme. The V_{max} value was more for expressed enzyme and less for immobilized which might be due to conformational changes in the enzyme.

4. **Conclusion**

Enzymes can replace the conventional catalysts in industrial application. The property of immobilizing enzymes on different materials can meet the required performance in enzyme catalysis. Hence the above study was shown to be a promising strategy for obtaining an active and stable lipase through successful immobilization using sodium alginate bead by entrapment method. The properties of immobilized lipase in terms of kinetic parameters makes them use in large scale industrial production of various products by binding to a wide range of substrates and can be reused for number of reactions.

5. **References**

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