

Applications of Immobilized Lipase enzyme produced from *Pseudomonas aeruginosa*.

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Abstract: Lipases (Triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the enzymatic hydrolysis of triacylglycerol into diacylglycerol & free fatty acids. Due to the wide range of applications of Lipases in various industries like Detergent, Leather, Textiles, Food, Pharmaceuticals, etc., Lipases serves as one of the most important enzymes in its class. The present study investigates the potentials of partially purified and immobilized extracellular Lipase enzyme produced from SP45 (*Pseudomonas aeruginosa*) isolated from oil contaminated soil samples for various applications. Immobilized SP45 Lipase was used for the enzymatic hydrolysis of edible oils and also for the detection of lipolysis of butter fats. Hydrolysis of edible oils reveals the fact that SP45 Lipase immobilized in sodium alginate beads exhibits maximum hydrolytic activity of 11.6667 U/ml/min against Groundnut oil when compared with other edible oils used in the study. As far as detection of lipolysis of butter fats was concerned, the copper soap test prominently showing bluish green zone around the colonies of SP45 (*Pseudomonas aeruginosa*) explains clear indication of strong lipolytic activity. In addition to that formation of clumps of butter fats were also observed which was caused due to the lipolysis reaction by immobilized SP45 Lipase enzyme. This clearly explains that immobilized SP45 Lipase enzyme exhibits the properties of lipolysis of butter fats.

Keywords: Lipase, *Pseudomonas aeruginosa*, Applications, Hydrolysis, and Lipolysis.

1. Introduction

In the recent years, the use of Lipase enzyme in various industrial applications has been grown tremendously and it is expected that these applications were in great demand in the recent future. Lipases were considered as one of the vital types of enzyme as it has versatility in the bioconversion reactions like esterification, transesterification, acidolysis, alcoholysis, aminolysis along with the traditional hydrolysis of triacylglycerides [1]. Lipases are one of the enzymes that are regularly used in various industries like Detergent, Food, Leather, Textiles, Pharmaceuticals, etc. [2]. Bacterial Lipases are the enzymes with tremendous demand due to their potential industrial applications & stability [3,4].

A lot of research has been done earlier on production of Lipase from microorganisms like *Bacillus spp.*, *Staphylococcus spp.*, *Candida spp.*, *Rhizopus spp.*, etc. both internationally as well as on national level [5-8]. Among all these, the *Pseudomonas spp.* remains considerably less explored for production of extracellular Lipase for various applications.

The present study gives us vital information regarding the efficient use of immobilized SP45 (*Pseudomonas aeruginosa*) Lipase enzyme in different applications like hydrolysis of edible oils and detection of lipolysis of butter fats.

2. Materials and Methods

In our previous studies around 99 lipolytic *Pseudomonas spp.* (SP01-SP50 and WP01-WP49) were isolated from 29 oil contaminated soil samples and 14 oil contaminated water samples collected from different regions of Mumbai, Solapur and Pune. It was also found that SP45 showed optimum Lipase activity (52.8867 U/ml) and thus SP45 was selected

for media optimization studies. Media optimization studies reveals the fact that SP45 (*Pseudomonas aeruginosa*) exhibits increased Lipase activity upto 66.2567 U/ml after optimization of various fermentation parameters like pH, temperature, carbon and nitrogen sources of fermentation medium [9,10].

The present study primarily aims at the applications of immobilized extracellular Lipase produced from SP45 (*Pseudomonas aeruginosa*) with the help of submerged fermentation for about 24 - 48 hours. Extracellular SP45 Lipase was produced with the help of ammonium sulphate precipitation followed by dialysis. The extracellular Lipase produced from SP45 (*Pseudomonas aeruginosa*) was then immobilized with the help of formation of sodium alginate beads as well as with ion exchange resins. SP45 Lipase immobilized with sodium alginate beads has showed greater Lipase activity than that of the ion exchange resins [11].

In the present study, immobilized SP45 Lipase was used for different applications like hydrolysis of edible oils & also for the detection of lipolysis of butter fats. The proposed study was carried out during the period commencing from October 2014 to February 2015.

2.1 Hydrolysis of Edible Oils

Hydrolysis of edible oils by immobilized SP45 Lipase (Sodium alginate beads) was performed by the method suggested by Zhiqiang Liu et al. with slight modifications.

Different edible oils like Groundnut oil, Sesame oil, Safflower oil, Coconut oil and Mustard oil were used in the present study. Briefly, 5ml of 0.1M Glycine NaOH Buffer (pH 8.5) and 4ml of 50% oil emulsion (2ml Propanol + 2ml Edible oil) were added to 100ml sterile flasks & the mixture was kept at 37°C for about 10-15 minutes. 3grams of sodium

alginate beads was then added to the mixture in one of the flask. In another flask, 3ml distilled water was added and this flask was treated as Blank. Then these flasks were kept on incubator shaker at 110rpm for about 30 minutes at 37^oC. After 30 minutes of incubation, 10ml of 95% Ethanol was added to the mixture immediately to cease the reaction. Liberated free fatty acids were then titrated with 0.05M NaOH using 1ml of Phenolphthalein as an indicator. One unit of hydrolytic activity of the Lipase was defined as the amount of enzyme which catalyzes the release of 1 μ mol of free fatty acids per minute under the assay conditions [12-14].

2.2 Detection of lipolysis of butter by immobilized SP45 Lipase

Detection of lipolysis of butter was performed using two different methods viz. copper soap test as described by M.V.Suryavanshi et al. with slight modifications and by using detection of lipolysis of butter fats.

2.2.1 Detection of lipolysis of butter by copper soap test

Overnight incubated culture of lipolytic SP45 *Pseudomonas aeruginosa* was streaked on the plate containing nutrient agar with melted unsalted butter. Then this plate was kept in an incubator at 37^oC for about 24 - 48 hours of incubation. After 48 hours of incubation, the petriplate containing streaked culture of SP45 *Pseudomonas aeruginosa* was then flooded with saturated solution of copper sulphate for about 10 - 15 minutes. The excess copper sulphate solution was then discarded and the plate was rinsed with autoclaved distilled water to remove unbound copper sulphate solution. Then the plate was observed for formation of bluish green streaks of insoluble copper soap around the colonies which indicates the lipolysis of unsalted butter [15].

2.2.2 Detection of lipolysis of butter fats by immobilized SP45 Lipase

Melted unsalted butter (50ml) was taken in two flasks and the flasks were labeled as Blank and Test. Immobilized SP45 Lipase in the form of sodium alginate beads (5gm) was added in the 'Test' flask while the other flask containing melted butter (50ml) was considered as 'Blank' as it contains sterile distilled water (5ml) instead of immobilized SP45 Lipase enzyme. Then these flasks were kept in an incubator shaker at 110rpm, 37^oC for 24 to 48 hours of incubation. After 24 and 48 hours of incubation, the flasks were checked for the presence of lipolysis of butter fats.

3. Results and Discussion

Immobilized Lipase enzyme can be used in various applications involving hydrolysis of triacylglycerol into glycerol and fatty acids. For the current study, hydrolysis of edible oils and detection of lipolysis of butter were used as applications of immobilized SP45 Lipase.

3.1 Hydrolysis of edible oils by immobilized SP45 Lipase

For hydrolysis of edible oils, different edible oils such as Groundnut oil, Sesame oil, Safflower oil, Coconut oil and Mustard oil were used. Although SP45 Lipase immobilized in sodium alginate beads showed the capability of hydrolysis

of all of the edible oils used in the study, SP45 Lipase shows maximum hydrolytic activity against Groundnut oil as shown in Table 1 and Figure 1.

Table 1: Hydrolysis of edible oils by Immobilized SP45 Lipase

Sr. No.	Edible Oils	Hydrolytic Activity (U/ml/min)
1	Groundnut oil	11.6667
2	Sesame oil	8.3889
3	Safflower oil	6.8333
4	Coconut oil	6.6667
5	Mustard oil	9.1667

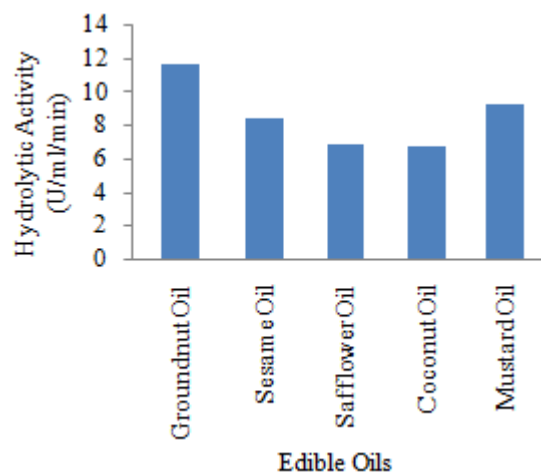


Figure 1: Hydrolysis of edible oils by Immobilized SP45 Lipase

3.2 Detection of lipolysis of butter by immobilized SP45 Lipase

The present study also emphasizes on the detection of lipolysis of butter by SP45 Lipase immobilized with sodium alginate beads as another application of immobilized Lipase enzyme.

3.2.1 Detection of lipolysis of butter by copper soap test

It was clearly observed that SP45 Lipase immobilized with sodium alginate beads exhibits the activity of lipolysis of unsalted butter as indicated in Figure 2. Copper soap test prominently showing bluish green zone around the colonies was an indication of strong lipolytic activity.



Figure 2: Detection of lipolysis of unsalted butter by Immobilized SP45 Lipase

3.2.2 Detection of lipolysis of butter fats by immobilized SP45 Lipase

It was observed that SP45 Lipase immobilized in sodium alginate beads exhibits the properties of lipolysis of butter fats. After 24 and 48 hours of shaking incubation, the flask containing immobilized SP45 Lipase alginate beads showed a clear lipolysis of butter fats when compared with the 'Blank' flasks which lack the SP45 Lipase enzyme as indicated in Figure 3.



Figure 3: Detection of lipolysis of butter fats by immobilized SP45 Lipase

4. Conclusion

In the present study, dialyzed SP45 Lipase enzyme immobilized in sodium alginate beads was used for the hydrolysis of edible oils. The hydrolytic activities of immobilized SP45 Lipase were studied with the help of different edible oils such as Groundnut oil, Sesame oil, Safflower oil, Coconut oil and Mustard oil. Although SP45 Lipase immobilized in sodium alginate beads showed the capability of hydrolysis of all of the edible oils used in the study, SP45 Lipase shows maximum hydrolytic activity against Groundnut oil. SP45 Lipase immobilized in sodium alginate beads exhibits hydrolytic activity of 11.6667 U/ml/min against Groundnut oil when compared with other edible oils used in the study.

For detection of lipolysis of butter two different strategies were used in the present study. Copper soap formation test gives a clear indication about lipolytic nature of SP45 (*Pseudomonas aeruginosa*) while other method used in this study showed lipolytic nature of SP45 Lipase immobilized with sodium alginate beads. The copper soap test prominently showing bluish green zone around the colonies of SP45 (*Pseudomonas aeruginosa*) explains clear indication of strong lipolytic activity. The present study also reveals the fact that SP45 Lipase immobilized in sodium alginate beads exhibits the properties of lipolysis of butter fats which was verified by the formation of clumps of butter fats due to the lipolysis reaction brought about by immobilized SP45 Lipase enzyme.

Presently, as biodiesel production serves as one of the most crucial areas of research due to environmental advantages and raised petroleum prices, thus there is an ample scope for further research on efficient use of immobilized SP45 Lipase in the production of Biodiesel which is the most essential demand of human mankind.

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