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# Study of Lipase Production by *Acinetobacter* sp. YMP

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**ABSTRACT**

The biosurfactant producing *Acinetobacter* sp. YMP which showed vegetable oil degradation potential on MSM was studied for lipase production. The production of lipase was confirmed on fat agar & lipase activity was measured by titrimetric method. The effect of environmental factors (agitation, time span , substrate concentration , pH & temperature ) on lipase production was studied by using *Acinetobacter* sp. YMP. The enzyme was further purified by Ammonium sulphate (60%) precipitation technique. The maximum lipase production by *Acinetobacter* sp. YMP was found to be within 120 hours at 450 rpm, 3.0 pH , 27° C temperature & at 2% substrate concentration. This species was also able to show upto 8 % oil degradation potential on MSM broth & agar.

**Key words:** Biosurfactant , MSM (Mineral salt medium) , Lipase.

## INTRODUCTION

The lipases (triglycerolacylhydrolases E. C. 3.1.1.3) are one of the most important biocatalysts catalysing the hydrolysis of triglycerides to glycerol & fatty acids & can carry out novel reactions in both aqueous and non aqueous media.[1] Among lipases of plants, animals and microorganisms[2], the microbial lipases find immense application, this is because microbes can be easily cultivated and their lipases can catalyse a wide variety of hydrolytic and synthetic reactions[3] .Lipases are useful in a variety of biotechnological fields such as food and dairy industry (cheese ripening, flavour development), paper manufacturing , detergent formulation, pharmaceutical preparations (naproxen, ibuprofen), agrochemicals industry (insecticides, pesticides) and oil processing chemical industries (fat and oil hydrolysis, biosurfactant synthesis)[4] & many newer areas. Each type of application requires unique properties with respect to specificity , stability, temperature & pH dependance. Many microorganisms like bacteria, fungi, yeast produce extracellular[5] lipases. Among these , the lipase produced by biosurfactant producing organisms shows high potential to degrade the hydrocarbons, oils and fats than the lipase produced by ordinary microorganisms. In addition , they show stability towards external environmental conditions like pH, temperature, substrate concentration etc. So we have studied production, purification & standardisation of lipase by biosurfactant producing organism *Acinetobacter* sp. YMP.

## MATERIALS AND METHODS

### Isolation of organism

Biosurfactant producing *Acinetobacter* species YMP was isolated from oily waste collected from Farsan industry located at Unchgaon , Kolhapur . It's cultural characteristics were studied & the species was confirmed by 16 S r RNA sequencing.

**Detection and confirmation of enzyme production of *Acinetobacter* sp. YMP**

A loopful of suspension of *Acinetobacter* species YMP was aseptically streaked on sterile egg yolk agar medium by four quadrant streaking method. The plates were incubated at room temperature for 24-48 hours. For confirmation of lipase activity, the organism was streaked on fat (butter) agar by four quadrant streaking method & the plates were incubated at room temperature for 24-48 hours.

**Study of enzyme lipase of *Acinetobacter* sp. YMP**

The selected isolate was inoculated in 100ml Medium A[6] with 1% groundnut oil concentration [(gm/lit) starch: 20; peptone: 20; $NH_4Cl$ : 3.8; $MgSO_4$: 1; $K_2HPO4$ : 5; groundnut Oil:1% at pH=7] and incubated for 24-72 hours on rotary shaker at 450 rpm at room temperature.

Then the cell free broth was obtained by centrifugation at 3500 rpm for 30 min and the supernatant was used as the crude enzyme for assay of lipase. The lipase activity was studied by titrimetric method [7] described by Umamaheshwari at regular intervals of 24, 48 and 72 hours.

**Study of effect of environmental factors on lipase production by *Acinetobacter* sp. YMP**

Various environmental factors affecting lipase production. ( e. g. agitation , time span , substrate concentration, $p^H$ & temperature.) were studied

**Effect of agitation on lipase production**

The isolate was inoculated in medium A & was incubated at static condition & on rotary shaker at 450 rpm at room temperature for 144 hours. The yield of lipase was determined by titrimetric method at regular intervals of 24, 48 , 72, 96, 120 & 144 hours.

**Optimization of substrate concentration for lipase production**

The isolate was inoculated in medium A with increasing substrate concentrations (groundnut oil) 0.5%, 1%, 1.5%, 2%, 2.5% on rotary shaker at 450 rpm for 120 hours. The optimum substrate concentration was determined by titrimetric method.

**Effect of pH on the lipase production**

The isolate was inoculated in medium A at different $p^H$ such as $p^H$ =1, 2, 3, 5, 7, 9 & 11 & incubated on rotary shaker at 4500 rpm for 120 hours & the maximum yield was determined by titrimetric method.

**Effect of temperature on the lipase production**

The isolate was inoculated in medium A at different temperatures such as 10°C, 27°C, 37°C, 55°C & incubated on rotary shaker for 120 hours & the maximum yield was determined by titrimetric method.

**Purification of lipase enzyme produced by *Acinetobacter* sp. YMP by precipitation method**

The organism was inoculated in medium A with 2% oil concentration at pH 3.0 and Temperature 27°C on rotary shaker at 450 rpm for 120 hours. Then equal amount of broth was added with increasing concentration of ammonium sulphate (10%, 20%....70%) and enzyme activity was detected by titrimetric method.

## RESULT AND DISCUSSION

The enzyme production of the isolate was detected by observing the zone of opalascence around the colonies on egg yolk agar plate & further confirmed by observing transluscent zone around the colonies on fat (butter) agar plates.

We studied the effect of various environmental factors (time span, agitation, substrate concentration , pH & temperature) on lipase production by *Acinetobacter* sp. YMP & the results of this study are as described in Table 1-4.

**Table 1: Agitation (rpm) Vs Enzyme units (mg/ml)**

| Time Span (Hours) | Enzyme Units (Mg/Ml) | |
|---|---|---|
| | Under static condition | Under agitated condition (450 rpm) |
| 24 | 0.1 | 0.13 |
| 48 | 0.14 | 0.25 |
| 72 | 0.2 | 0.28 |
| 96 | 0.22 | 0.3 |
| 120 | 0.2 | 0.32 |
| 144 | 0.18 | 0.28 |

Thus, the maximum yield of enzyme was obtained within 120 hours under agitated condition (450 rpm), at 2 % substrate cocentration, at 3 pH & at 27$^0$ C.

Lipase enzyme was purified by precipitation with ammonium sulphate in increasing concentration. The maximum precipitation and enzyme activity was observed at 60% ammonium sulphate concentration .

**Table 3: pH Vs Enzyme units (mg/ml)**

| pH | Enzyme units(mg/ml) |
|---|---|
| 1 | 0.30 |
| 2 | 0.42 |
| 3 | 0.54 |
| 5 | 0.33 |
| 7 | 0.30 |
| 9 | 0.03 |
| 11 | 0.005 |

**Table 2: Substrate concentration (%) Vs. Enzyme units (mg/ml)**

| Substrate concentration (%) | Enzyme units (mg/ml) |
|---|---|
| 0.5 | 0.14 |
| 1 | 0.32 |
| 1.5 | 0.32 |
| 2 | 0.39 |
| 2.5 | 0.26 |

**Table 4: Temperature °C Vs Enzyme units (mg/ml)**

| Temperature $^0$ C | Enzyme units(mg/ml) |
|---|---|
| 10 | 0.18 |
| 27 | 0.47 |
| 37 | 0.24 |
| 55 | 0.19 |

## CONCLUSION

By using biosurfactant producing *Acinetobacter* sp. YMP , enzyme lipase was produced. It's activity was determined by titrimetric method. Also , optimum conditions for enzyme production were determined. The enzyme was further purified by ammonium sulphate precipitation method. Thus , lipase produced by biosurfactant producing *Acinetobacter* sp. YMP can be exploited in various areas of Industrial Microbiology and Biotechnology.

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