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Production of extracellular lipase by *Serratia marcescens* isolated from industrial effluent

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KEYWORDS

Lipase, *Serratia marcescens*, Optimization; Pnpp; Industrial Effluents.

ABSTRACT

Lipase production was carried by *Serratia marcescens*. Studies were undertaken to improve lipase production by optimizing pH, incubation time, and temperature. The effect of carbon source was studied by adding glucose, fructose, sucrose, lactose, mannitol, glycerol, starch, groundnut meal and soya meal to a medium containing nitrate and other mineral source. The best activity of 6.102 U/ml after 45 hour at 30 ^{o} C at pH 7 was obtained in the media supplemented with glucose and starch. Peptone, soyatone, tryptone, casein, yeast extract, beef extract, ammonium nitrate, ammonium chloride, ammonium sulphate and sodium nitrate were tested as nitrogen source in this medium, with casein giving a lipolytic activity of 8.441 U/ml after 45 hour, the highest yield obtained in this study. Lipolytic activity yield was high in gingly oil about 8.595 U/ml at pH 7 after 45 hour incubation than other substrate source. Incubation time, pH and temperature of the medium were same in all the case.

Introduction

Lipolytic enzymes are currently attracting an enormous attention because for their biotechnological potential (Benjamin and Pandey, 1998). Microbial lipases are among the most important group of enzymes with applications in detergents, manufacture of food ingredients and pitch control in the pulp and paper industry. These enzymes are also interesting as biocatalyst in organic media, for transesterification reactions, synthesis of chiral compounds, etc. (Jaeger and Reetz, 1998). Industrial scale extraction of lipases is carried out in bacteria, fungi, actinomycetes and cultures of plant and animal cells (Ako *et al*., 1995). Most of the well studied microbial lipases are inducible extracellular enzymes. They are synthesized within the cell and exported to its external surface or environment

(Fukumoto *et al*., 1963; Ota *et al*., 1982). Consequently, the genes of many bacterial lipolytic enzymes have been identified, cloned, expressed and enzymes have been characterized (Jaeger *et al*., 1999). Bacterial lipases are glycoproteins, but some extra cellular bacterial lipases are lipoproteins (Macre and Hammond., 1985). Amongst the lipase-producing organisms, *Bacillus, Candida, Penicillium, Pseudomonas, Rhizomucor* and *Rhizopus* spp. are the outstanding ones (Rapp and Backhaus, 1992).

Among bacteria, *Achromobacter, Alcaligenes, Arthrobacter, Pseudomonas*, *Staphylococcus* and *Chromobacterium* spp. have been exploited for the production of lipases. But in recent years, *Serratia* sp. has been studied for its ability to produce lipase (Abdou, 2003). The present report concerns the isolation and production media optimization of extracellular lipase by *Serratia marcescens*.

Materials ad Methods

Sample Collection

For the present study, effluent sample was collected from different oil mills like groundnut oil, palm oil and coconut oil in a sterile container for the isolation of lipase producing organisms under laboratory condition.

Isolation of Lipolytic Microbes

For the isolation of lipolytic microbes, 1.0 gm of sample was mixed in 100 ml of double distilled water. It was then serially diluted (10^{-1} to 10^{-6}) and the diluted samples were placed on tributyrin agar plates. The formation of clear zone around the colony on the plate was considered as lipolytic microbes.

Identification

Microbes which formed large clear zone around the colony were identified based on morphological, biochemical and physiological characters according to Bergey's manual of determinative bacteriology (Holt *et al*., 1996) which was identified as *Serratia marcescens* and was maintained on nutrient agar slant supplemented with 1% olive oil.

Production Media Composition

The original liquid medium contained (per liter) Olive oil 5%, peptone 5gm, yeast extract 5gm, glucose 5gm, NaCl 0.25 gm and $MgSO_4$. $7H_2O$ 0.5gm which acts as standard. Different chemical and physical parameters were optimized using the standard production media

Optimization of pH

The standard production medium was adjusted to different pH ranges from 4 to 10 using 0.1 N Hcl and 0.1N NaOH, respective organisms was inoculated to check the optimum pH and its effect on lipase production.

Optimization of Incubation Temperature

The standard production medium was inoculated and incubated at temperatures ranging from 4 to 60°C to test for their effect on lipase production and the optimum temperature for maximum lipase production.

Optimization of Incubation Period

The production medium was incubated under standard conditions for a time period of 15 to 70 hrs individually on the organisms to test the effect of time in the production of lipase.

Optimization of Carbon Source

The effect of carbon source on lipase production was studied using fructose, lactose, sucrose, glucose, starch, mannitol, glycerol, groundnut meal and soyameal which were substituted in standard production media.

Optimization of Nitrogen Source

For the increased production of lipase enzyme by *Serratia marcescens* various nitrogen sources were typically supplemented in standard production medium by replacing with organic and inorganic nitrogen sources like peptone, soyatone, yeast extract, tryptone, beef extract, casein, ammonium chloride, ammonium nitrate, ammonium sulphate and sodium nitrate.

Optimization of Substrate Source

By using different substrates sources such as neem oil, palm oil, pongemia oil, ground nut oil, soyabean oil, sun flower oil, olive oil, seasame oil, castor oil, hippe oil, mustard oil, coconut oil, gingly oil and cod liver oil, their effect on lipase production was assessed at optimum pH, incubation temperature and time.

Enzyme Assay

Lipase assay was carried out using tributyrin agar plate assay as qualitative test to detect lipase activity (Samad *et al*., 1989)

Lipase Activity

Lipase activity was determined by ρNPP (ρ-nitrophenyl palmitate) method (Winkler and Stuckmann, 1979). The coefficient of extinction of ρ-nitrophenol (ρNP), $1.5 \cdot 10^4$ L/mol/cm, was determined from the absorbance measured at 410 nm of standard solution ρNP. One unit was defined as the amount of enzyme liberating 1µmol of ρ-nitrophenol per minute at 37 ^{o}C.

Result and Discussion

Enrichment culture technique enabled the isolation of strains from oil mill effluent with lipolytic activity in tributyrin agar plate. The lipolytic microbes were further screened and characterized by their features and reactions and then identified as Gram negative, rod shaped motile organisms (Table 1). Finally morphological and biochemical test indicated that the suspected organism was *Serratia marcescens*

The efficiency of lipase activity was analyzed ρNPP assay for different pH, temperature, incubation time, carbon, nitrogen and substrate sources as shown in (Figure 1a, d, c, d, e and f).

Result shows that highest lipase activity for *S. marcescens* was achieved at pH 7 at 30°C for incubation period of 45 hour in the media containing starch and glucose as carbon source, casein as nitrogen source and gingly oil as substrate source.

The production of extracellular lipase in submerged culture of *Serratia rubidaea* has been investigated. The lipase production was optimized in shake flask experiments.

Figure.1a Effect of pH on Lipase Activity

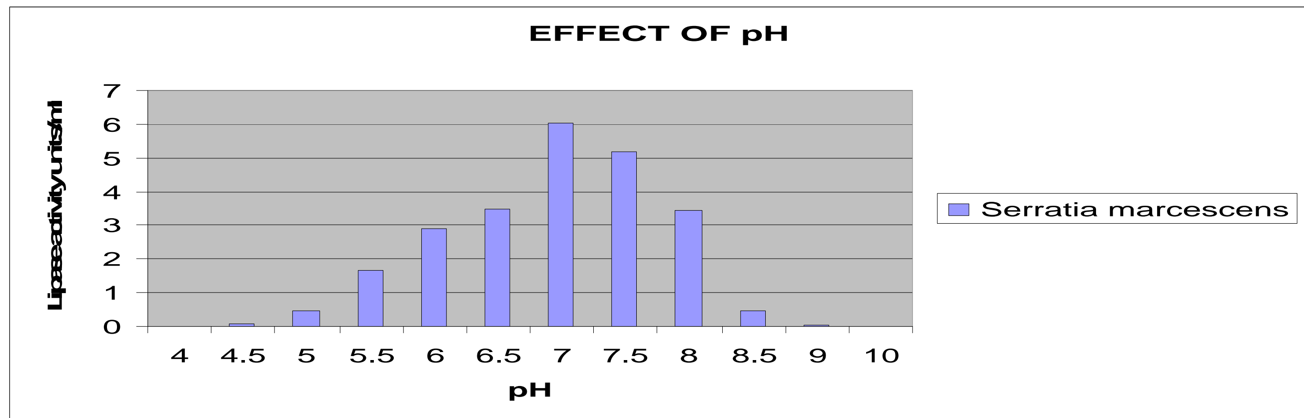


Figure.1b Effect of Temperature on Lipase Activity

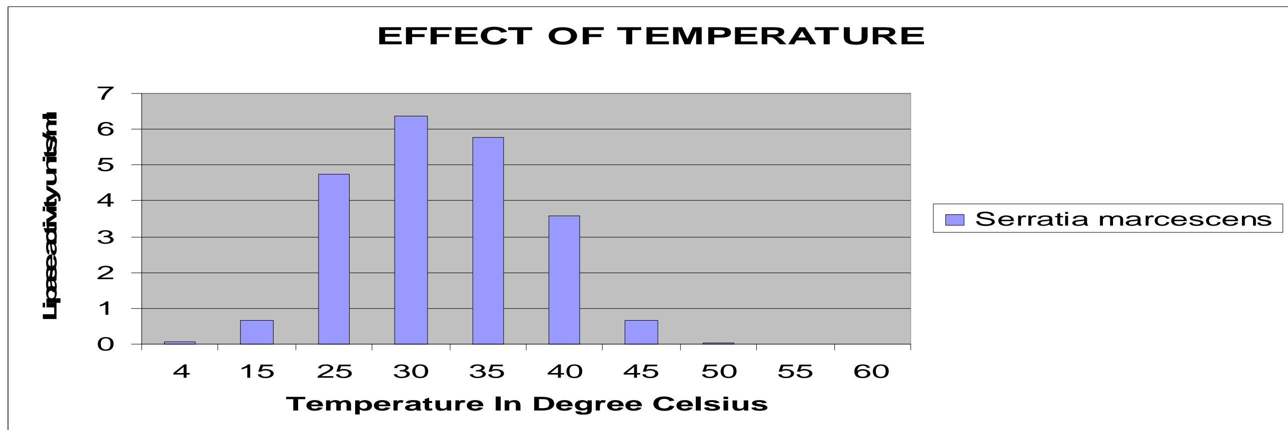


Figure.1c Effect of Incubation Time on Lipase Activity

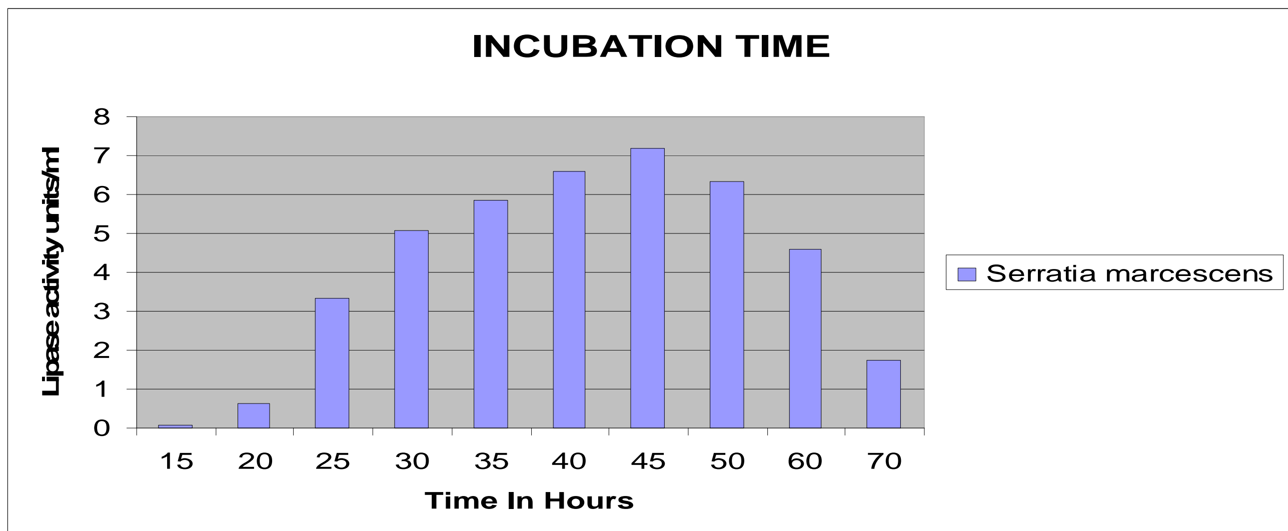


Figure.1d Effect of Carbon Sources on Lipase Activity

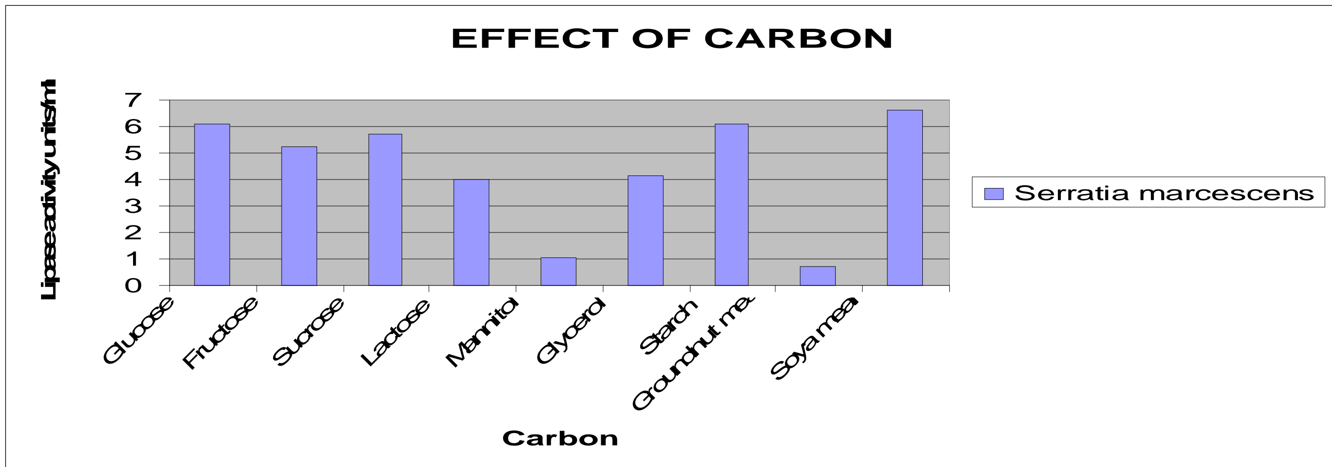


Figure.1e Effect of Nitrogen Source on Lipase Activity

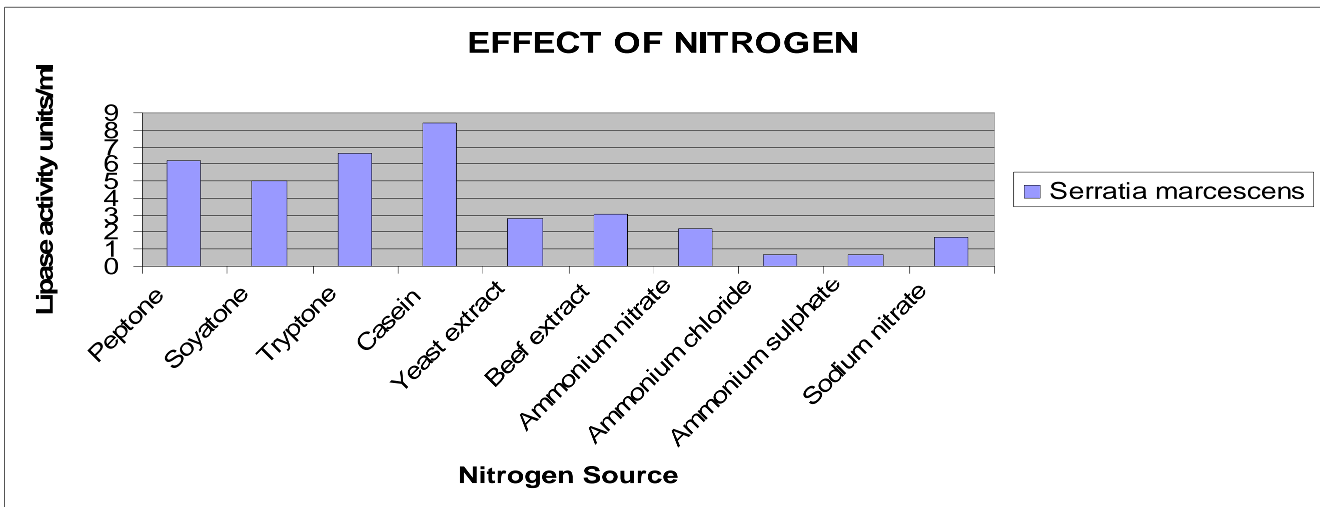
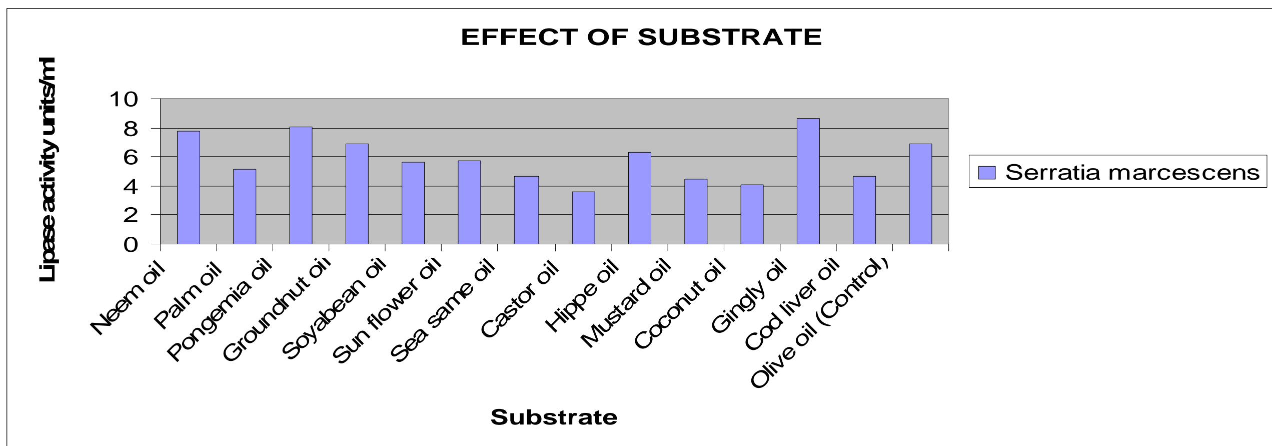


Figure.1f Effect of Substrate Sources on Lipase Activity



The observed pH and temperature range optimum for maximum lipase production were 7-8 and 30-40°C (Grasian *et al*., 2008). The lipase production by *Serratia marcescens* prefers neutral pH (6.5-7) and Pseudomonas aeruginosa MB prefers neutral pH (Gao *et al*., 2004; Marcin et al., 1993). The present study revealed that lipase production by *S. marcescens* was high in pH 7. The lipase activity by *Serratia marcescens* was found to be maximum at pH 8 (Abdou 2003) *B. thermocatenulatus* and *B. stearothermophilus* produce lipase with similar properties. Their molecular mass is approx. 45 KDa and they display maximal activity at pH 9 and 65 ^{o} C (Schmidt et al., 1996; Kim et al., 1998), but in the present study highest lipase activity for *S. marcescens* was achieved at 30 ^{o}C for incubation period of 45 hour.

In the present study different carbon sources were screened for their efficiency to support lipase production. Among the tested, *S. marcescens* results maximum lipase activity in the media containing *glucose* as carbon source. Takahiro et al., (1988) achieved lipase production by a fed-batch culture of *Pseudomonas fluorescens*. During the cultivation, temperature, pH and dissolved oxygen concentration. Olive oil was used as a carbon source for microbial growth.

A number of factors affecting the production of extracellular lipase by Cryptococcus sp. S-2 were investigated. Consecutive optimization of nitrogen, carbon sources and inducers enhanced lipase activity and under optimum conditions the lipase activity was 65.7 U/ml of the culture medium in 120 h at 25 and at pH 5.6. Sardine oil, soy bean oil and triolein were effective inducers for lipase production (Kamini *et al*., 2003). Among the nitrogen sources, casein produced maximum lipase compared to others. This is because casein is a simple milk protein and can be easily utilized by a candidate species.

In the present study gingly oil show maximum lipase activity as substrate source. Supachok *et al*., (2001) found highest activity with ρ-nitrophenyl ester-caprate as the synthetic substrate and tricaprylin as the triacylglycerol.

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