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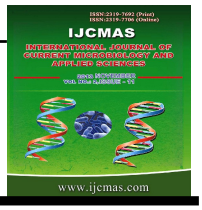

Original Research Article

Comparative Studies on the production of Lipase by *Bacillus* species under various growth parameters

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Keywords

Lipase; *Bacillus subtilis; Bacillus licheniformi; Bacillus amylo-liquefaciens*; Effluents.

A B S T R A C T

Bacteria producing extracellular lipase was isolated from industrial effluents and identified as *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens* by morphological, biochemical and Molecular characteristics. Growth of the organisms and lipase production were measured with varying pH (4-10), incubation temperature (4, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60^{o} C), incubation time, various nitrogen, carbon and substrate sources. Growth of the organism and lipase activity was maximum at pH 7.5 after 60 hours of incubation time at 45^{o} C for *Bacillus subtilis*, at pH 8 after 70 hour incubation at 50 ^{o} C for *Bacillus licheniformis* and pH 8.5 after 50 hour of incubation at 45 ^{o} C for *Bacillus amyloliquefaciens*. Among carbon sources groundnut meal resulted in maximum of 8.424 U/ml in Bacillus *subtilis*, 8.617 U/ml in *Bacillus licheniformis* and *Bacillus amyloliquefaciens* in starch showed 7.291 U/ml in of lipase activity, among nitrogen source *Bacillus subtilis* showed high result in tryptone 5.931 U/ml, *Bacillus licheniformis* 6.915U/ml and *Bacillus amyloliquefaciens* resulted in 6.460 U/ml high lipase activity in casein. *Bacillus subtilis resulted in* 6.921 U/ml in soyabean oil, *Bacillus licheniformis* in neem oil showed activity of 8.339 U/ml and groundnut oil resulted 6.506 U/ml for *Bacillus amyloliquefaciens* as substrate .This present work describes the comparative study on the effect of different growth media and conditions on lipase production by three *Bacillus*.sp.

Introduction

Lipases find applications in many areas of biotechnology due to their ability to catalyze enantioselective reaction with a wide range of substrates and their stability over wide variations of temperature and pH. Lipids constitute a large part of the earth's biomass and lipolytic enzymes play an important role. Their major application in terms of quantity is as additives for laundry detergent (Pandey *et al*., 1999; Sharma *et al*., 2001). They are the major industrial enzymes extensively used in pharmaceuticals, textiles, food, medical and chemical industries (Salleh *et al*., 1999). One of the most promising fields of lipases application is in the production of

optically active compounds for the agrochemical and pharmaceutical industries (Rasor and Voss, 2001; Zaks and Dodds, 1997). Lipases display a high degree of specificity and enantio selectivity for esterification and transesterification reactions (Okahata *et al*., 1995). Industrial scale extraction of lipases is carried out in bacteria, fungi, actinomycetes and cultures of plant and animal cells. Among them, microbes are metabolically versatile and hence have advantage in many industrial processes leading to the development of microbial biotechnology (Ako *et al*., 1995; Heraldson *et al*., 1995). Considering the importance of lipase enzyme, three lipase producing Bacillus.sp (*B.subtilis, B.licheniformis and B. amyloliquefaciens*) have been isolated and optimized in the present study.

Materials and Methods

Isolation of Lipolytic Microbes

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. For the isolation of lipolytic microbes, 1.0 gm of sample was dissolved in 100 ml of double distilled water. It was then serially diluted (10^{-1} to 10^{-6}) and the diluted samples were plated 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 were identified as *Bacillus subtilis B.licheniformis* and *Bacillus amyloliquefaciens*, the isolated organisms were 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 were inoculated to check the optimum pH and its effect on lipase production.

Optimization of Incubation Temperature

The standard production medium was inoculated with *Bacillus subtilis*, *B.licheniformis* and *B.amyloliquefaciens* incubated at temperatures ranging from 4 to 60°C to test their effect on 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 *Bacillus subtilis*, *B.licheniformis* and *B.amyloliquefaciens* various nitrogen sources were typically supplemented in standard production medium by replacing 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. 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 ^oC.

Result and Discussion

Enrichment culture technique enabled the isolation of strains from soil sample 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 positive, rod shaped motile organisms (Table 1). Finally morphological and biochemical test indicated that the suspected organisms were *Bacillus subtilis, Bacillus licheniformis* and *B. amyloliquefaciens.* The efficiency of lipase activity was compared among three *Bacillus.sps* by ρNPP assay method with different pH, temperature, incubation time, carbon, nitrogen and substrate sources as shown in (Figure 1a, d, c, d, e and f).

The results of the present study showed that highest lipase activity for *Bacillus subtilis* was found at pH 7.5, for *Bacillus licheniformis* at pH 8 and for *Bacillus amyloliquefaciens* at pH 8.5 .In case of incubation temperature maximum lipase activity was analyzed in *Bacillus subtilis* and *Bacillus amyloliquefaciens* at 45 ^oC, whereas in *Bacillus licheniformis* activity was high at 50 ^oC. After 60 hour incubation *B. subtilis* resulted in high activity, *B. licheniformis* after 70 hour of incubation and *Bacillus amyloliquefaciens* after 50 hour of incubation. Among carbon source, groundnut meal resulted in maximum activity in both the species and starch in case of *Bacillus amyloliquefaciens*. *B. subtilis* showed high

Fig.1a Effect of pH on Lipase Activity.

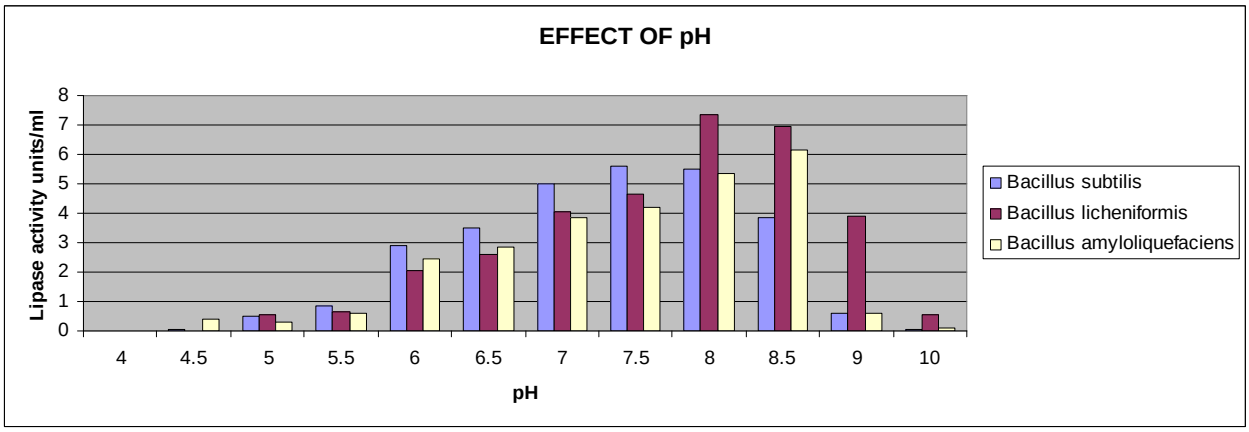


Fig.1b Effect of Temperature on Lipase Activity

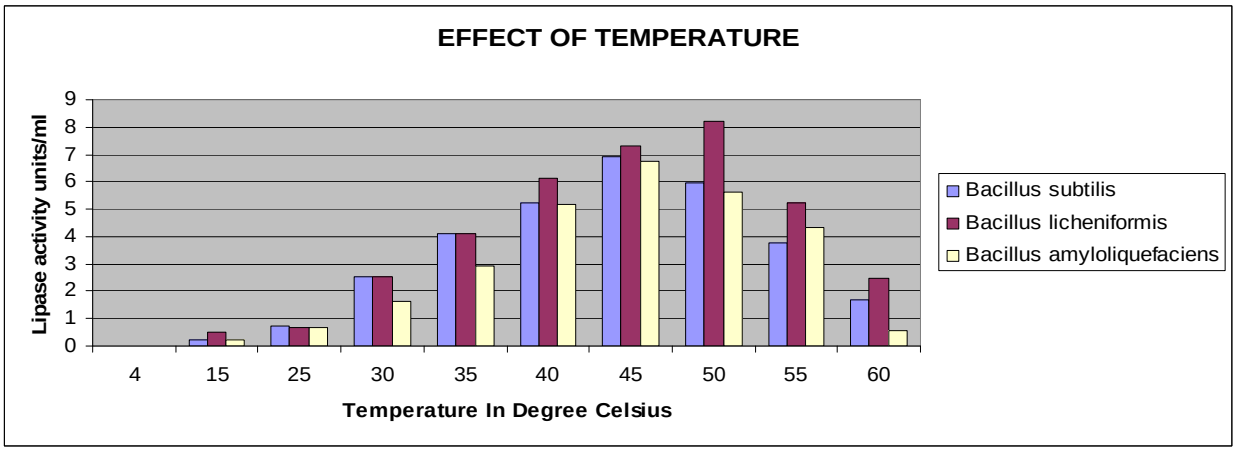


Fig.1c Effect of Incubation Time on Lipase Activity.

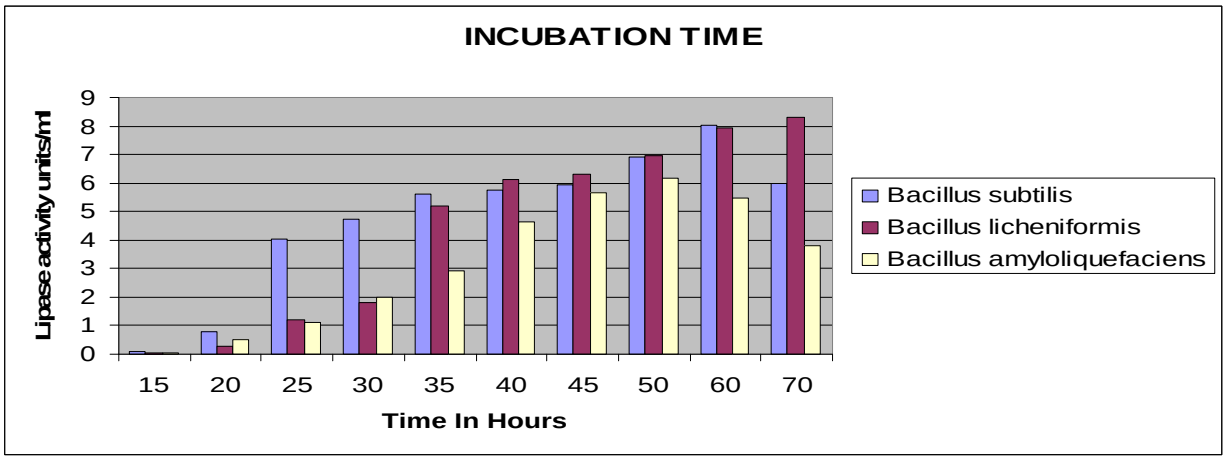


Fig.1d Effect of Carbon Sources on Lipase Activity

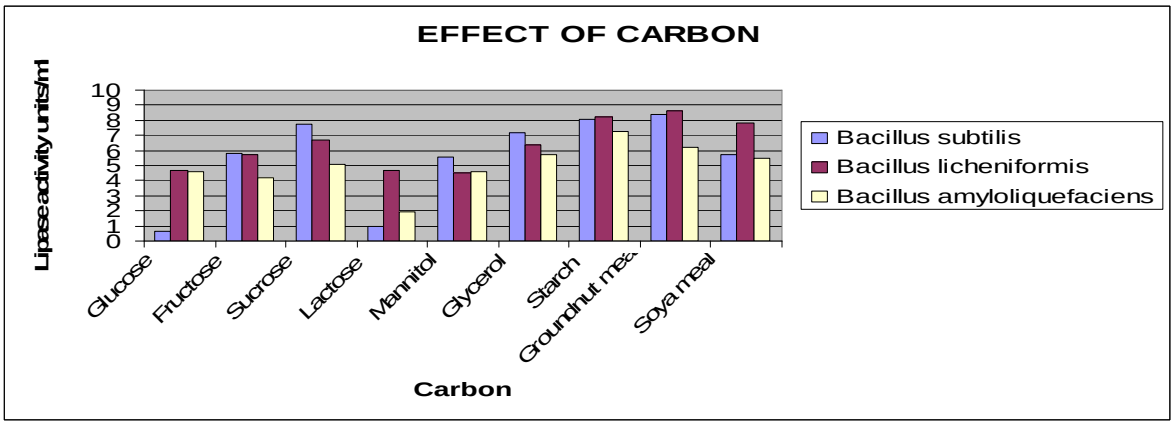


Fig.1e Effect of Nitrogen sources on Lipase Activity

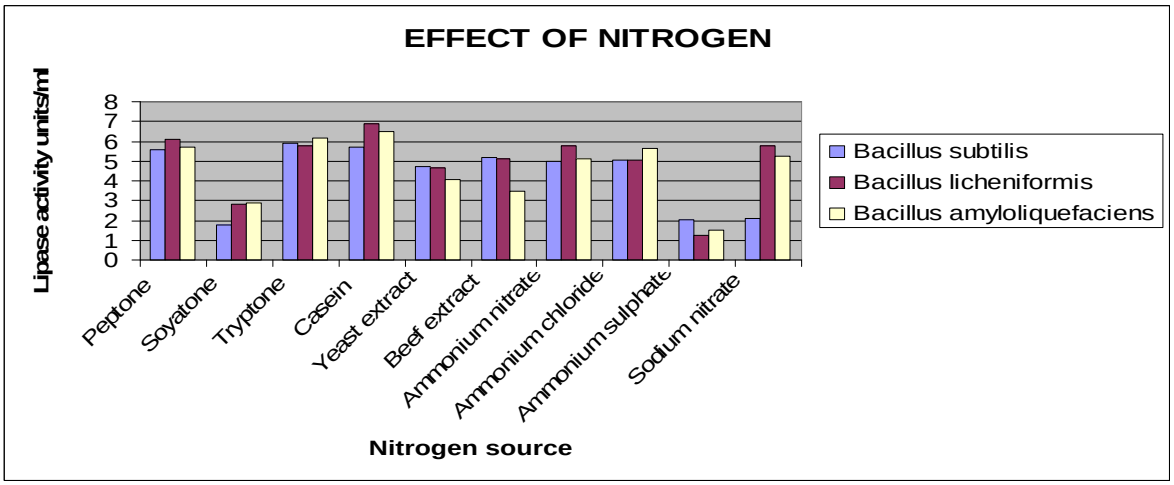
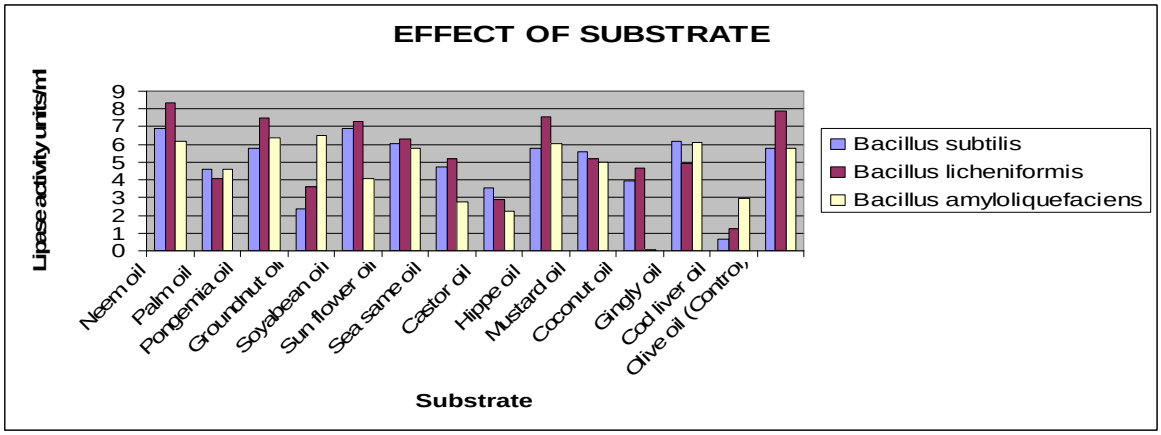


Fig.1f Effect of Substrate sources on Lipase Activity



lipase activity in tryptone, *B. licheniformis* and *Bacillus amyloliquefaciens* activity high in casein as nitrogen source. Soyabean oil showed maximum lipase activity in *B. subtilis*, neem oil for *B. licheniformis* and groundnut oil showed high results for *Bacillus amyloliquefaciens*. Comparision of all the parameters between the three organisms showed highest lipase activity was achieved in *Bacillus licheniformis.*

The presence of large hydrolysis haloes after incubation of strains *Bacillus* sp. BP-6 and *Bacillus* sp. CR-179 on tributyrin indicates both strain code for atleast one lipolytic enzymes (Dartois *et al*., 1992). The extracellular lipase producing bacterium was isolated from an oil mill refinery effluent, which was identified as *B. subtilis and B.licheniformis.* The maximum lipase production by *B. licheniformis* was at room temperature in peptone medium and the activity was higher in triglycerides than in esters (Sangiliyandi and Gunasekaran, (1996). In the present study, the lipase producing bacterial strains were isolated from groundnut oil mill effluent and identified as *Bacillus subtilis, Bacillus licheniformis* and *B.amyloliquefaciens* . The maximum lipase production by *B. licheniformis* was at 50 ^{o}C after 70 hour of incubation in media containing groundnut meal, casein and neem oil.

In the present study, the influence of medium temperature indicated that the lipase production by the isolated strains was higher at 45 ^{o}C for *Bacillus subtilis* and *B. amyloliquefaciens* and 50 ^{o}C for *B. licheniformis* when compared to other. According to Kamini *et al*., (2000) have reported that the lipase activity of *Staphylococcus*.sp was maximum at 37 ^{o}C. A lipase activity was detected on whole cell and in the culture supernatant. The highest activity was expressed at 45 ^{o}C and pH 6.8 (Dupuis *et al*., 1993), in this study lipase activity was high in the medium having pH 7.5 for *Bacillus subtilis*, for *Bacillus licheniformis* was pH 8 and *B.amyloliquefaciens* at 8.5

Consecutive optimization of nitrogen, carbon sources and inducers enhanced lipase activity under optimum conditions. Sardine oil, soy bean oil and triolein were effective inducers for lipase production (Kamini *et al*., 2000). In this study, groundnut meal results maximum activity in both the species as carbon source and starch in *B. licheniformis B. subtilis* results enhanced lipase activity in tryptone, *B. licheniformis* and *B. amyloliquefaciens* casein as nitrogen source. Soyabean oil results maximum lipase activity in *B. subtilis*, neem oil for *B. licheniformis* and groundnut oil result in *Bacillus amyloliquefaciens*

According to Selva Mohan *et al*., (2008) indicated that the lipase production varied between *Bacillus* strains and also between varying parameters tested. The maximum lipase production was recorded at pH 7 during 24 h of the culture period by *Bacillus* strain B5. Acoording to Lakshmi *et al*., (1999), reported that the production of lipase was high in medium added with vegetable oil than the medium added with glucose.

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