International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Evaluation of Growth Parameters on Extracellular Lipase Enzyme Production by *Staphylococcus Aureus*

Prasad. M. P

Department of Microbiology/Biotechnology, Sangenomics Research Lab, Domlur Layout, Bangalore 560071, India

Abstract: ***Lipases have the ability to perform biotransformation which has made them increasingly popular in the detergent, food, chemical, and pharmaceutical industries. Environmental pollution due to oil effluents is one of the major problems and there is need for detoxification and destruction of these contaminants effluents. Different Lipase producing microorganisms are used for the oil effluent remediation process to detoxify and degrade the oil effluents. In the present investigation, bacteria producing extracellular lipase were isolated from industrial effluents and identified as Staphylococcus aureus by morphological, biochemical and Molecular characterization. Growth of the organisms and lipase production were measured with varying pH (4-10), incubation temperature (4 to 60^{o} C), incubation time, various nitrogen, carbon and substrate sources. The composition of the medium and changes in pH, temperature and incubation period affected the amount of enzyme produced. Lipase activity was maximum at pH 7 for incubation period of 48 hours at 35^{o} C and results in highest lipid activity in the media containing glycerol as carbon, casein as nitrogen and cod liver oil as substrate source. The present work describes the comparative study on the effect of different growth media and conditions on lipase production by Staphylococcus aureus.***

Keywords: *Staphylococcus aureus*, Lipase enzyme, Industrial effluent, Optimization

1. Introduction

Lipases are serine hydrolases of considerable physiological significance and industrial potential that can catalyze numerous reactions such as hydrolysis, interesterification, esterification, alcoholysis and aminolysis [1, 2]. Lipases are used in the dairy and food industries, in the production of flavor and aroma components, in oleochemical industry and in medical applications. Microbial lipases are used to obtain PUFAs from animal and plant lipids such as menhaden oil, tuna oil, and borage oil. Free PUFAs and their mono- and diglycerides are subsequently used to produce a variety of pharmaceuticals including anticholesterolemics, antiinflammatories, and thrombolytics [3]. 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 [4, 5]. Microbial lipases are the most important group of biocatalysts used for a variety of different biotechnological applications [6, 7]. Interest in *Staphylococcus aureus* lipase dates from the work of [8] who associated the egg yolk factor containing lipase activity with virulence. Staphylococci secrete extracellular enzymes whish may have evolved a scavenging enzymes, degrading polymeric material in the environment. These may provide a survival advantage by releasing low molecular mass compounds for growth [9]. Lipase of *Staphylococcus epidermidis* 9 was purified from culture supernatant fluid. Two polypeptides (51 and 43 kDa) were detected by SDS-PAGE, of which the 43 kDa polypeptide reacted with anti-lipase serum [10]. *Staphylococcus aureus* produces an extracellular lipase in a synthetic medium, the composition of the medium affected the amount of enzyme produced [11]. The present work describes the effect of different media and growth conditions on lipase production by *Staphylococcus aureus.*

2. Materials and Methods

2.1 Sample Collection

For the present study, effluent soil 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.

2.2 Isolation of Lipolytic Microbes

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. 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, which was identified as *Staphylococcus aureus* and was maintained on nutrient agar slant supplemented with 1% olive oil.

The standard 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, *Staphylococcus aureus* 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 *Staphylococcus aureus* 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
Different substrate (Inducers) sources such as neem oil, palm oil, pongemia oil, ground nut oil, soyabean oil, sun flower oil, seasame oil, castor oil, hippe oil, mustard oil, coconut oil, gingly oil and cod liver oil, with olive oil as control, their effect on lipase production was assessed at optimum pH, incubation temperature and time.

Enzyme Assay and activity
Lipase assay was carried out using tributyrin agar plate assay as qualitative test to detect lipase activity [12]. Lipase activity was determined by ρNPP (ρ-nitrophenyl palmitate) method [13]. 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°C.

3. Result

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 positive, cocci in clusters and motile organisms (Table-1). Finally morphological and biochemical test indicated that the suspected organism was *Staphylococcus aureus* Figure-1. The lipase activity was analyzed by ρNPP assay for different pH, temperature, incubation time, carbon, nitrogen and substrate sources as shown in (Figure-2 to 6).

Figure 1: *S.aureus* showing lipolysis on Trybutrine agar

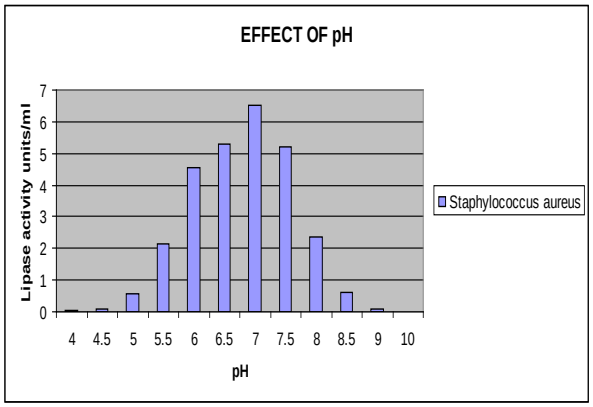

Figure 2: Effect of pH on Lipase Activity

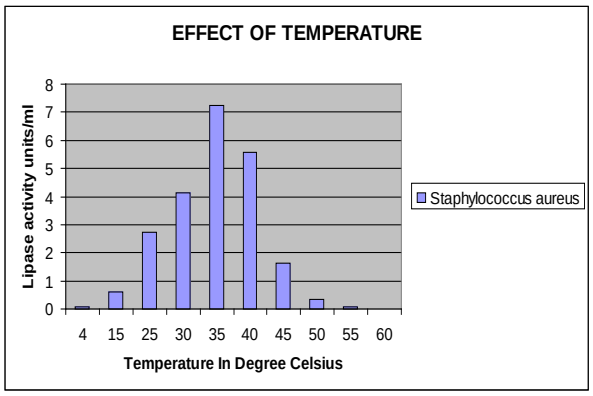

Figure 3: Effect of Temperature on Lipase Activity

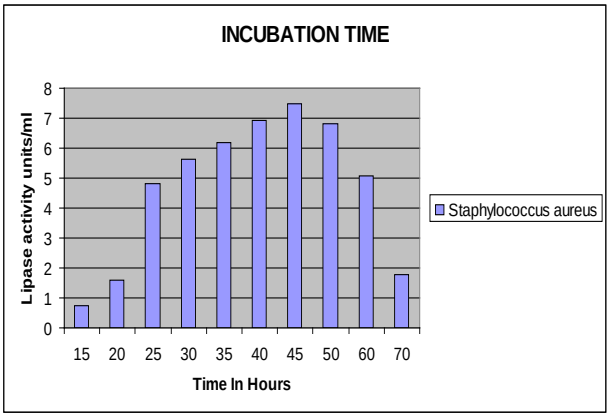

Figure 4: Effect of Incubation Time on Lipase Activity

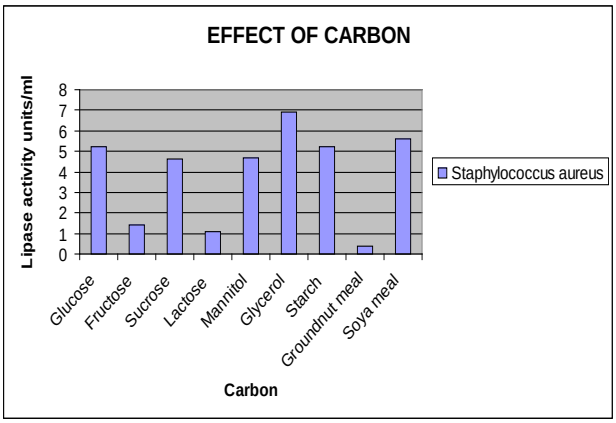

Figure 5: Effect of Carbon Sources on Lipase Activity

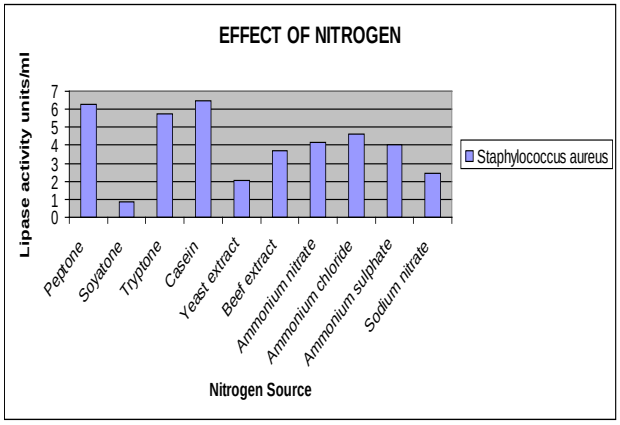

Figure 6: Effect of Nitrogen Source on Lipase Activity

Results show that highest lipase activity for *S. aureus* was achieved at pH 7 at 35^{o}C for incubation period of 45 hour in the media containing glycerol as carbon source, casein as nitrogen source and cod liver oil as substrate source.

4. Discussion

The current work reports on the initial characterization of the production of lipase by a strain of *Staphylococcus aureus.* Lipase formation by *S.aureus* was shown to be constitutive. Lipids added to the medium inhibited the enzyme synthesis, as was found to be the case with *Pseudomonas fragii* [14], whereas lipids stimulated lipase production by fungi [15, 16]. 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 [17]. A thermostable lipase produced by a thermophilic *Bacillus* sp. J33 was purified to 175-fold with 15.6% recovery by ammonium sulphate and Phenyl Sepharose column chromatography. Extracellular lipases are produced by a variety of microorganisms: fungi, yeasts, and bacteria, including actinomycetes. The lipase genes have been cloned from fungi, a yeast, Pseudomonas species, and Staphylococci. In the present study, maximum lipase activity of the culture medium was seen after 45 hours at 35^{o} C and at pH 7. Glycerol, casein and cod liver oil were effective inducers for lipase production. According to [18], 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. According to [19], reported that the production of lipase was high in medium added with vegetable oil than the medium added with glucose.

High concentration production of an extracellular enzyme lipase was achieved by a fed-batch of *Pseudomonas fluorescens*. During the cultivation, temperature, pH and dissolved oxygen concentration were maintained at 23 ^{o} C, 6.5 and 2-5 ppm, respectively. Olive oil was used as a carbon source for microbial growth [20].

Extracellular lipase produced by Micrococcus sp. ML-1 was studied for its applicability in the common used detergents for the removal of oily stains from various types of fabrics. The crude lipase was used in combination with various commonly used detergents [21]. Lipase activity produced during cultivation of *Fusarium oxysporum f*.sp. vasinfectum in shake flasks was predominantly extracellular. Only a small amount was found in or on the mycelium. The fungus required peptone for significant lipase production. Addition of trimyristin, olive oil, span 85 and oleic acid to growing shake-flask cultures, and the addition of olive oil and oleic acid to suspensions of washed mycelium [22].

References

[1] Bornscheuer, U.T., Bessler, C., Srivinas, R and Krishna, S.H (2002). Optimizing lipases and related enzymes for efficient application. *Trends Biotechnol*, 20: 433-437
[2] Pandey, A., Benjamin, S., Soccol, C. R., Nigam, P., Krieger, N and Soccol, V.T (1999). The realm of microbial lipases in biotechnology.*Biotechnol.Appl.Biochem*, 29:119-131
[3] Jaeger, K.E. and Eggert, T. (2002). Lipases for biotechnology.*Curr.Opin.Biotechnol*, 13 390-397
[4] Ako, C., Cillard, D and Jennings, B.H (1995). Enzymatic modification of trilinolein. Incorporation of N-3Saturated fatty acid. *J. Am.Oil Chem. Soc*, 72: 1317-1321
[5] Heraldson G.G., Gudmundsson, B.O and Almarsson, O (1995). The synthesis of homogeneous triglycerides of eico sapenbaenoic acid and decosahetaenoic acid lipase. *J.Tetrahedron*, S1: 941-952
[6] Macrae, A.R and Hammond, A.R (1985). Present and future applications of lipases. *Biotechnol Genet Eng*, 3: 193-217
[7] Rosenau, F and Jaeger, K.E (2000). Bacterial lipases from *Pseudomonas*: regulation of gene expression and mechanisms of secretion. *Biochimie*, 82: 1023-1032
[8] Gillespie, W.A and Alder, V.G (1952). Production of opacity in egg yolk by coagulase positive staphylococci. *J.Path. Bact*, 64:187
[9] Arvidson, S.O (1983). Extracellular enzymes from *Staphylococcus aureus. In Staphylococci and Staphylococcal Infections*, 2: 745-808
[10] Angela M. Farrell., Timothy J. Foster and Keith t. Holland (1993). Molecular analysis and expression of the lipase of *Staphylococcus epidermidis*. *J. General Microbiology*, 139: 267-277

[11] Mates, A and Sudakevitz, D (1973). Production of Lipase by *Staphylococcus aureus* under Various Growth Conditions. *J. Appl. Bact*, 36: 219-226
[12] Samad, M.Y.A., Razak, C.N.A., Salleh, A.B., Yunus, W.M.Z., Ampton, K and Basri, M (1989). A plate assay for primary screening of lipase activity, *J. Microbiol Methods*, 9: 51-56
[13] Winkler, U.K and Stuckmann, M (1979). Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. *J. Bacteriol*. 138, 663-670
[14] Smith, J.H and Alford, J.A (1966). Inhibition of microbial lipase by fatty acids. *Appl. Microbiol*, 14: 699
[15] Ota, Y., Suzuki, M and Yamada, K (1968). Lipids and related substances inducing the lipase production by *Candida paralipolytica*. *Agr. Biol. Chem*, 32: 390
[16] Yosidha, F., Motai, H and Icshima, E (1968). Effect of lipids on production of lipase by *Torulopsis ernobi*. *Appl. Microbiol*, 16:845
[17] Kamini, N.R., Fujii, T., Kurosu, T and Iefuji, H (2000). Production, purification and characterization of an extracellular lipase from the yeast, Cryptococcus sp. S-2. Process Biochemistry, 36: 317-324
[18] Selva Mohan, T., Palavesam, A and Immanvel, G (2008). Isolation and characterization of lipase-producing *Bacillus* strains from oil mill waste. *African Journal of Biotechnology*, 7**(15)**: 2728-2735
[19] Lakshmi, B., Kangueane, P., Abraham, B and Pennatheu, G (1999). Effect of vegetable oils in secretion of lipase from *Candida rugosa* 9DSM2031. *Lett. Appl. Microbiol*, 29: 66-70
[20] Takahiro Suzuki., Yoshinao Mushiga., Tsuneo Yamane and Shoichi Shimizu (1988). Mass production of lipase by fed-batch culture of *Pseudomonas fluorescens*. *Appl Microbiol Biotechnol*, 27: 417-422
[21] Naz Neeru, J.K. Gupta and Gupta, L.K (2001). Application of Micrococcal Alkaline Lipase in Commonly Used Detergents. *Indian Journal Microbiology*, 41: 177-179
[22] Peter Rapp (1995). Production, regulation and some properties of lipase activity from Fusarium oxysporum f. sp. vasinfectum. *Enzyme and Microbial Technology*, 17: 832-838.