Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7560

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2019) *7*(5), 476-481

Research Article

Indian Journal of Pure & Applied Biosciences

Isolation and Medium Optimization of Bacterial Lipase from Oil Mill

Krishna Patel * and Samir Parikh

Sir P.T. Science College, Modasa, Gujarat

*Corresponding Author E-mail: krishnapatel9330@gmail.com

Received: 10.06.2019 | Revised: 17.07.2019 | Accepted: 24.07.2019

ABSTRACT

Lipase (glycerol ester hydrolases EC 3,1,1,3) is a important biocatalyst because of their demand in various industrial application. In this study lipolytic bacteria isolate KK12 Bacillus spp. was isolate from oil contaminated sample by using tributyrine agar medium. Isolate KK12 bacillus spp gave the highest zone of hydrolysis with 25mm. Lipase production was improved by optimized for different oils, carbon sources, nitrogen sources, substrate, pH, temperature, metal ions. The highest lipase activity was 14U/l at 37^{0}C at pH 7.6. In this study isolate KK12 Bacillus spp giving best activity with peptone was 9.2U/l. Lipase activity was high in olive oil about 9.8U/l at 37^{0}C after 48hours incubation than other substrate. Other different parameters like pH, temp., metal ions also study in this research same all of these.

Keywords: *Lipase, Optimization, Bacterial isolates, Lipase activity, Oil contaminated soil*

INTRODUCTION

Lipase are one of the biocatalysts with potential for contributing to the multibillion dollar underexploited lipid technology and used in many industrial application Lipase are hydrolytic enzyme. They hydrolyze triglycerols in to free fatty acid, diacylglycerols and glycerol. They also gives other activities like phospholipase, isophospholipase, cholesterol esterase, amidase, cutinases etc. Lipase contain unique properties like their substrate specificity, temperature, pH, activity in organic solvents, etc (031). So, lipase have been studied for many years and also produced on a large scale. Lipase are important enzyme for biotechnology and industrial applications such as food, dairy, detergent, pharmaceutical, agrochemical, cosmetic, oleochemical and also used for diagnostic tool etc (Verma et al., 2012, Jaeger & Reetz, 1998).

In environment many sources are available that produce lipase like plants, animals human pencriase, bacteria, fungi, and yeast In which bacteria, fungi and yeast are very cheapest sources of lipase. There are s examples of bacteria produce lipase like pseudomonas spp. Bacillus spp. Alcalgenes spp., Staphylococcus spp. Serratia spp. etc. some fungi produce lipase like Aspergillus spp., Rhizopus spp., Penicillium spp. Etc. and some yeast like Candida spp. etc. For lipase production from m.o. both submerged and solid state fermentation are used since 2004. In present study we concern the isolation of lipase from oil contaminated soil.

Cite this article: Patel, K., & Parikh, S. (2019). Isolation and Medium Optimization of Bacterial Lipase from Oil Mill, *Ind. J. Pure App. Biosci. 7*(5), 476-481. doi: http://dx.doi.org/10.18782/2320-7051.7560

Then production of lipase by submerged fermentation and media optimization of lipase by isolated Bacillus spp.

MATERIALS AND METHODS

Sample collection

For present study isolation of lipolytic bacteria oil contaminated soil sample was collected from different areas like Vikas oil industries, Talod (Sabarkantha), Gujarat Pramukh enterprise, Dugarwada, Modasa (Aravalli), Gujarat. Sample was collected in sterile polythene bags and these bags was stored in refrigerator in 4^0 C for laboratory study.

Isolation lipase producing bacteria

Take a 1 gm of oil contaminated soil sample was mixed in 100 ml distilled water. It was serially diluted (10^{-1} to 10^{-6}) and spread on tributyrene agar plates. Incubate it at 37^0C for 48 hours.After incubation observed the formation of clear zone of lipid hydrolysis around the bacterial colony on plates.

Maintainance of isolated bacteria

The isolated lipase producing bacteria was transferred on nutrient agar slant for maintainance and revived after every months.

Identification

The selected isolated lipase producing bacteria was identified based on morphology, biochemical and physiological character according to Bergey's manual of determinative bacteriology

Lipase enzyme production

The composition of production medium used was: (%g/l) peptone 0.2 gm, NH4H2PO4 0.1gm, Nacl 0.25gm ,$MgSo_4.7H_2O$ 0.04gm, Cacl2.2H2O 0.04 gm, Olive oil 2.0(v/v), Tween 80 1to 2 drops as emulsifier, pH- 7.0^9, 005 Overnight cultures were suspended in 5 ml D/W and used as the inoculums. Submerged culture was incubated in 500ml Erlenmeyer flask containing 100ml of liquid medium on a rotary shaker (150rpm) and incubated at 37^0C. After 24 hours of incubation the culture was centrifuged at 10,000 rpm for 15 min at 4^0 C. The cell free supernatant was used as the extracellular enzyme. The lipase activity in the supernatant was determined by the spectrophotometric method. The isolate that produce maximum lipase was selected for further study

Lipase activity

The sample collected from culture broth were centrifuged for 15 min. at the speed of 10,000 rpm and supernatant was assayed for extracellular lipase activity. Lipase was determined specrophotometrically by using phenyl acetate substrate.

Using phenyl acetate as substrate for lipase activity and determined by librated phenol by Folin Ciocalteu reagent. 2.4 ml of phenyl acetate 165 µM in Tris HCL buffer, 0.1 M and pH 7, with 1% Triton X-100) and 0.1 ml lipase is incubated at 40^0C during 10 minutes and absorbance was measured at 750nm (Mohamed et al., 2013).

Optimization of fermentation conditions

Effect of pH

Study the effect of pH of the fermentation medium on lipase production was performed by varying pH of the medium from 4 to 14 using 0.1N HCL & 0.1N NaOH.

Effect of Temperature

Study effect of incubation temperature of the fermentation medium on lipase production was performed by temperature varying from18^0C to 60^0C

Effect of Carbon source

Effect of carbon source for lipase production was performed with different carbon sources like glucose, lactose, fructose, starch, Dextrin, were inoculated in production media and incubate at 37^0C and measured lipase activity.

Effect of Nitrogen source

For the increased production of lipase various nitrogen sources were used by replacing with inorganic and organic nitrogen sources such as peptone, yeast extract, soyabean, tryptone, ammonium chloride. These different nitrogen sources were added in production media and check lipase activity.

Effect of substrate

Effect of various substrate is study by using substrate like olive oil, Ghee, Coconut oil, groundnut oil, sunflower oil and seasum oil inoculated in production medium and cheack the lipase activity.

Effect of metal ions

The production medium was inoculated with various metal ions to study the effect of metal ions on lipase production metal ions added like $Mg^{+2,}Cu^{+2,}Ca^{+2,}Ba^{+2},K^{+}$.

RESULT AND DISCUSSION

Soil is a natural reservoir of microbial population (Veerapagu et al., 2003). Isolation of lipase producing bacteria from these soil sample can be useful for obtaining bacterial species for potential of production lipase enzyme lipase producing bacteria have been isolated from vegetative oil contaminated soil. In present study Soil sample was collected from different areas of Talod and Modasa, Gujarat. Lipase producing bacteria isolated by using tributyrine agar plate assay. Lipolytic activity was found by observing clear zone of lipid hydrolysis around the colony.46 bacteria give lipolytic activity, among the isolates KK12 gave highest hydrolytic zone produced was selected for further study which was Gram positive, big rods, spore forming bacillus sp. With 25mm zone of lipid hydrolysis.

The efficiency of lipase activity was analyzed by using spectrophotometric method using phenyl acetate. This assay was used for optimization of fermentation media for highest production of lipase. Different physical and chemical parameters were used.

Effect of pH

The pH of the medium adjusted to different pH like 4,5,6,7,7.6,8,9,10,11,14 and incubated at 37^0C for 48 hours. The enzyme production varied from 0.7 U/l to 10.2U/l. Isolate KK12 gave optimum lipase production at pH 7.6 and activity is 10.2U/l. when the pH was increased from 7.6 to 14 the lipase production was decrease. Maximum lipase production was observed at pH 7 in Serratia marscens, pseudomonas spp (Veerapagu, et al., 2003, Gao et al., 2004), Staphylococcus spp.

Effect of temperature

The optimum temperature for lipase production of isolate KK12 was found 37^0C showing lipase activity was 10.5U/ml. The enzyme production was decreased while temperature was increased above 37^0C. The observed pH and temperature range optimum for maximum lipase production were 7-8 and 30^0C-40^0C

Effect of carbon source

Maximum lipolytic activity was observed in production medium 10.7U/l with glucose, 2.5U/l with lactose, 5.8U/l with fructose, 2.4U/l with starch.2.5U/l with dextrin. Many other bacterial sp. Like staphylococcus and pseudomonas sp. give highest activity in glucose use as a carbon source (Veerapagu et al., 2003, Devayani et al., 2014).

Effect of nitrogen source

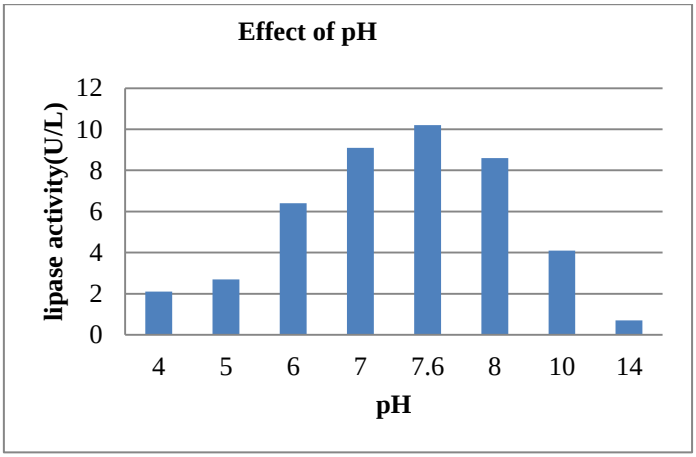
In the present study different nitrogen source were used for lipase production. Maximum lipase activity of KK12 was given in media containing peptone (9.2U/l) also Achromobacter xylosoxidans (TSMCN) was gave highest lipase activity in peptone containing media.

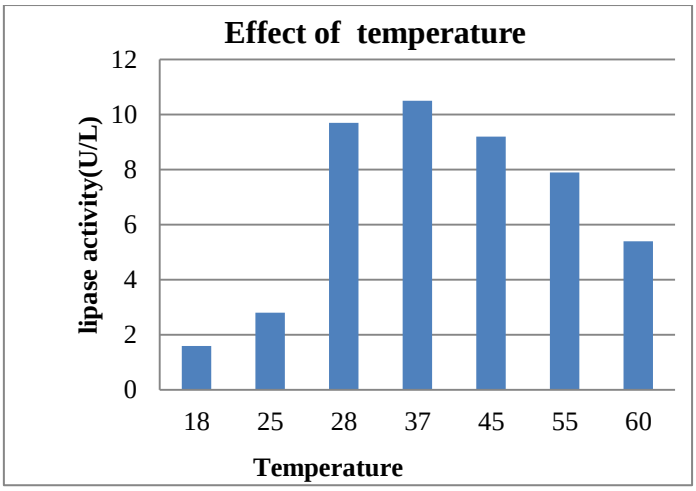
Effect of substrate

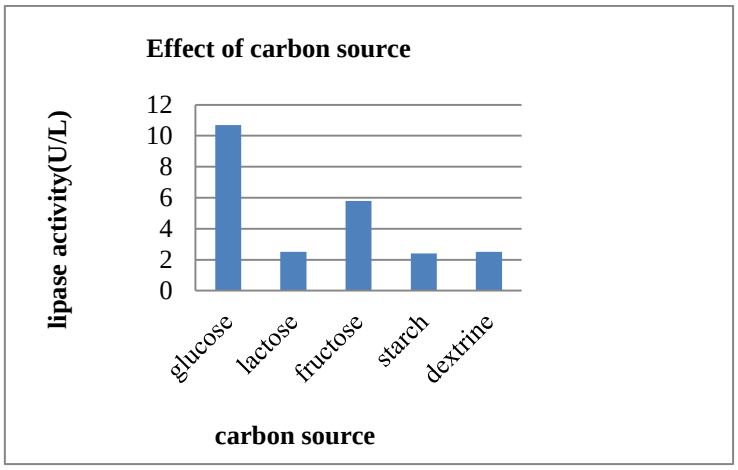
Various oils were used for the production of lipase as an inducer. In the present study maximum lipase production occur in olive oil (9.8U/l) followed by palm oil (4.4U/l), ghee(2.8U/l), coconut oil(1.7U/l), groundnut oil(8.6U/l), sunflower oil(5.4U/l) seasum oil (2.5U/l).

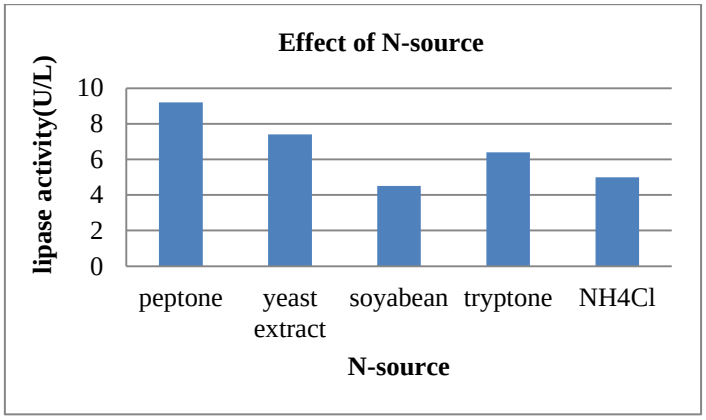
Effect of metal ions

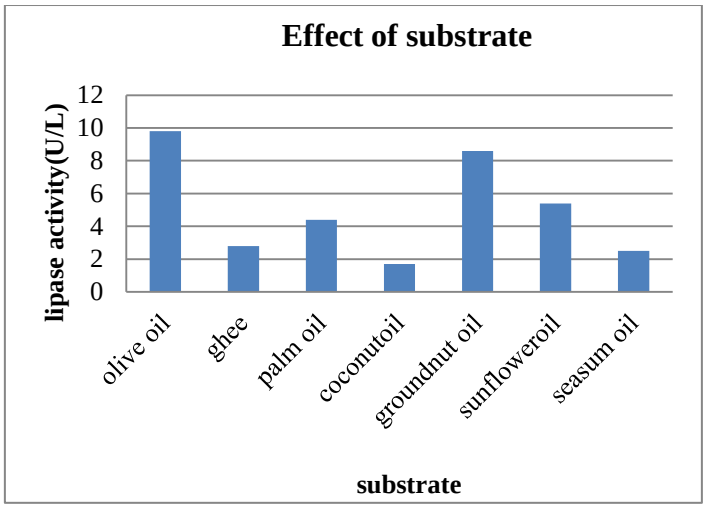
Metal ions can either stimulate and inhibit microbial lipase production. Ca^{+2} ions play important role in the structure and function of lipase enzyme. While $Zn^{+2,}Fe^{+2},Fe^{+3}$ ions inhibit its activity (Verma et al., 2012), 031.different metal ions were used for lipase production in which highest activity occur in Mg^{+2} ions with 12.8U/l.

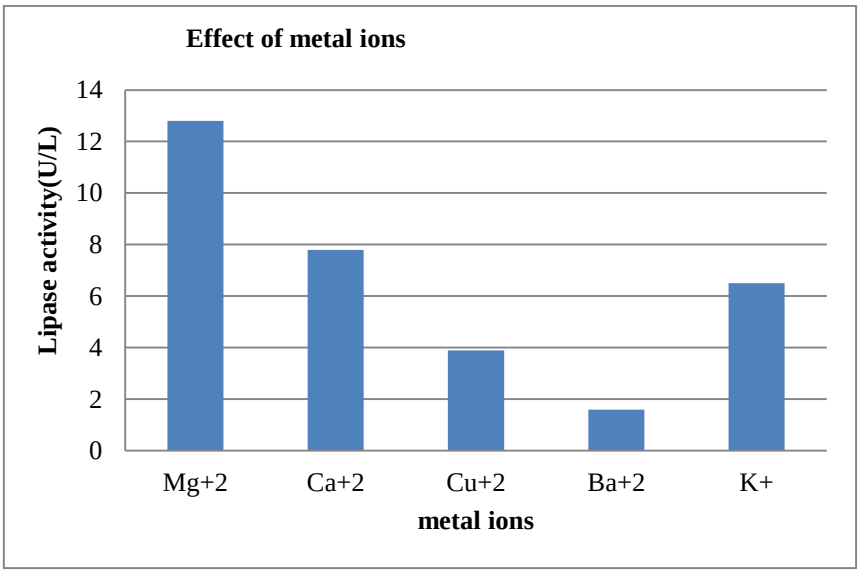

Effect of pH
lipase activity(U/L)
12
10
8
6
4
2
0
4
5
6
7
7.6
8
10
14
pH


Effect of temperature
lipase activity(U/L)
12
10
8
6
4
2
0
18
25
28
37
45
55
60
Temperature


Effect of carbon source
lipase activity(U/L)
12
10
8
6
4
2
0
glucose
lactose
fructose
starch
dextrine
carbon source







CONCLUSION

In the present study gives the information for the optimization of culture condition of the isolate K12 identified as bacillus for lipase production. The condition such as pH, temperature, carbon source, nitrogen source, substrate, metal ions. These results show that highest lipase produce at 37^0C temperature and pH -7.6. In this study olive oil show maximum activity with peptone as a nitrogen

source and in the presence of Mg^{+2}. The optimized condition for lipase production developed in this study can be used for a large scale production of lipase.

REFERENCES

Abdou, A.M. (2003). Purification and partial characterization of psychrotrophic serratia marscence lipase. *J. Dairy Sci*, *86*, 127-132.

Ako, C., Cillard, D., & Jennings, B.H. (1995). Enzymatic modification of trilinolein. Incorporation of N- 3Saturated fatty acid. *J. Am.Oil Chem. Soc*, *72*, 1317-1321.

Benjamin, S., & Pandey, A. (1998). Candida rugosa lipases: Molecular biology and versatility in biotechnology. Yeast. *14*, 1069-1087.

Devayani, R., Tipre, M., Purohit, S., & Shailes, R. (2014). Dave. Production and characterization of lipase frm Staphylococcus sp. SDMlip. International Jouranal of Current and Applied Sciences. Vol-3, 423-436.

Fukumoto, J., Iwai, M., & Tsujisaka, Y. (1963). Studies on lipase. Purification and crystallization of a lipase secreted by Aspergillus niger. *J. Gen. Microbiol*, *9*, 353-361.

Gao, L., Xu, J.H., Li, X.J., & Liu, Z.Z. (2004). Optimization of Serratia marcescens lipase production for enanotioselective hydrolysis of 3- phenyl glycidic acid ester. *J.Ind. Microbiol. Biotechnol*, *31*, 525-530.

Jaeger, K.E., & Reetz, M.T. (1998). Microbial lipases form versatile tools for biotechnology. *Trends biotechnol*. *16*, 396-403.

Jaeger, K.E., Dijkstra, B.W., & Reetz, M.T. (1999). Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases. Annu. *Rev. Microbiol*, *53*, 315- 351.

Veerapagu, M., Sankara N., Onmurugan, A.K., & Jeya, K.R. (2003). Screening Selection Identification Production and Optimization of Bacterial Lipase From Oil Spilled Oil. *Asian Journal of Pharmaceutical and Clinical Research*. 6.

Mohamed A. Abd-Elhakeem, Ahmed M. Elsayed, Taher, A., & Alkhulaqi. (2013). New Colorimetric Method for Lipases Activity in Microbial Media. *American Journal of analytical chemistry*. *4*, 442-444.

Sonia Dhiman & Shilpa S Chapadgaonkar. Optimization of lipase production medium for a bacterial isolate. *International Journal of Chem tech Research*. *5*, 2837-2843.

Verma, N., Takur, S., & Bhatt, A. K. (2012). Microbial lipase: Industrial Applications and Properties (A Review).*International jouranal of biological science*. *1*, 88-92.

Winkler, U.K., & Stuckmann, M. (1979). Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by Serratia marcescens. *J. Bacteriol*. *138*, 663-670.