INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

SCREENING AND ISOLATION OF LIPASE PRODUCING *PSEUDOMONAS SP.* FROM OIL CONTAMINATED SOIL

Amol A. Shirsath *[1], Pradnya S. Vishwe [2], Vivek S. Vishwe [3]
[1]JSPM's Charak College of Pharmacy and Research, 720/1&2, Wagholi, Pune-nagar Road, Pune, Maharashtra, India
[2]SCSSS's Sitabai Thite College of Pharmacy, Pune Nagar Bypass, Shirur, Pune, Maharashtra, India
[3]Sirmaxo Chemicals Pvt. Ltd., MIDC Tarapur, Boisar, Thane, Maharashtra, India
*Corresponding Author Email: aas.ccopr@gmail.com

Article Received on: 15/12/18 Approved for publication: 22/01/19

DOI: 10.7897/2230-8407.1003100

ABSTRACT

Pseudomonas sp. are found everywhere in the environment. *Pseudomonas sp.* are considered to be the most resistant and nuisance causing bacteria. Several *Pseudomonas sp.* have been shown to produce lipases. Leakages from pipelines, underground storage tanks of gas stations, improper disposal of petroleum wastes and stranded oil spills are the major sources of surface and groundwater contamination. The techniques used to remove hydrocarbons from such contaminated sites are quite expensive. In this study, we tried to find out the presence of oil degrading *Pseudomonas sp.* from oil contaminated sites. Various soil samples from oil contaminated sites were incubated in *Pseudomonas sp*. selective cetrimide broth. The enriched culture was screened for the presence of lipase producing organisms onto tributyrin agar. Further biochemical tests were performed for the confirmation of genus Pseudomonas.15 lipase producing microorganisms were obtained from 9 different soil samples. Isolate no- 8 was found to be the highest lipase producing *Pseudomonas sp.* Presence of oil in the nutrient medium serves as a switch to produce lipase. As the enzyme lipase degrades oil and a fat, its production is higher in the organisms present in the oil contaminated soil. Lipase producing *Pseudomonas sp.* can be effectively used for the bioremediation of oil contaminated sites.

KEY WORDS: *Pseudomonas sp.,* Cetrimide, Tributyrin, Lipase

INTRODUCTION

Lipases (triacyl glycerol acyl hydrolases, EC 3.1.1.3) constitute a diverse family of enzymes that initiate the catabolism of fats and oils by hydrolyzing the fatty acylester bonds of acylglycerols. Lipases are widely distributed in nature and have been found in many species of animals, plants, bacteria yeast and fungi. Although their wide distribution, the enzymes from microorganisms are most interesting because of the ease of handling the microorganisms, simple processes and their potential application in various industries ranging from the use in the laundry detergent to stereospecific biocatalysts.[1,2,3,4]

Pseudomonas sp. are found everywhere in the environment. They are present in air, water; soil as well as there are pathogenic strains which cause diseases like cystic fibrosis. Several *Pseudomonas* have been shown to produce lipases. Some of them have been sequenced i.e. *Pseudomonas fragi, P. cepacia* and *P. aeruginosa*. The enzyme of Pseudomonas sp. Strain ATCC 21808 is an attractive lipase. It is stable at high temperatures (50-60 ºC) and over the broad pH range (pH 5 to 10).[5]

Pseudomonas sp. are considered to be the most resistant, degradative and nuisance causing bacteria. Many operations in the petroleum exploration, production and transportation have the potential to affect the environment in different degrees. In this study, we have tried to investigate the lipase producing ability of *Pseudomonas sp.* isolated from oil contaminated soil. Hence, it can be used as one of the easy and efficient techniques of bioremediation.[6,7]

MATERIAL AND METHODS[7,8,9]

Collection of soil sample

9 Soil samples were collected in the clean autoclaved bottles from 10 different sites in Maharashtra. The soil samples were mostly collected from the sites contaminated with oil or from the roots of oil seeds producing trees.

Enrichment of the soil sample

Approximately 2 gm of soil sample was inoculated in 100 mL of cetrimide broth for selective cultivation of *Pseudomonas sp.* Cetrimide broth inoculated with soil sample was incubated for 24-48 hours at 37ºC.

Screening of bacteria

The above enriched culture was serially diluted and plated onto Tributyrin agar. The colonies showing zones of clearance around them after incubations were streaked onto the *Pseudomonas* (For Pyocyanin) agar for the confirmation of genus.

Identification of bacteria

Identification of Pseudomonas sp. was carried out using Gram staining and various biochemical tests such as sugar broth fermentation, nitrate reduction test and catalase test.

Selection of highest lipase producing *Pseudomonas sp*

All cultures were maintained on Nutrient agar slants. All the isolates were suspended in standard saline and the optical density of the suspensions was set to approximately 0.1 with the help of Mac Ferland standard tube of 0.5 value. 5 µL of each suspension was spot inoculated on a large tributyrin agar plate containing

Rhodamine - B and the plate was incubated for 48-72 hours at 37ºC. Culture showing maximum zone of clearance around the colony was selected.

RESULTS

The enriched sample when spread on the tributyrin agar, showed the presence of zone of clearance around some colonies. Only the Pseudomonas sp. were able to grow on Pseudomonas agar. Among the 15 isolates, isolate no.- 8 was showing maximum zone of clearance around the colony.

Gram nature and morphology

The isolated bacterium was found to be Gram negative short rods in appearance.

Colony characteristics of Pseudomonas isolate no. 8 on Nutrient agar are as follows. (Table 1).

Table 1: Colony characteristics of Pseudomonas isolate

Colony Characters	Results
Shape	Circular
Size	1-2 mm
Colour	Creamy
Margin	Smooth
Opacity	Translucent
Elevation	Slight
Consistency	Sticky
Pigment	Green

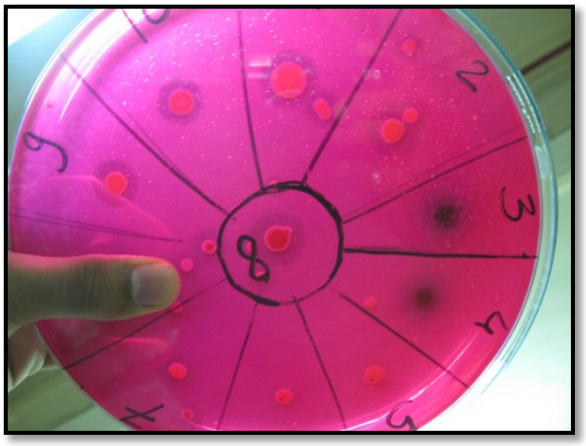

Figure 1: Screening of lipase producing *Pseudomonas sp.* on Tributyrin agar containing Rhodamine – B

Biochemical tests[10]

1. **Sugar broth fermentation:** (Table 2).

2. **Nitrate Reduction Test**

Pink colour developed immediately when sulphanilic acid reagent and α- naphthylamine reagent were added on the growth of the bacteria on Nitrate agar slants.

3. **Catalase Test**

Bubble formation was observed immediately after adding Hydrogen peroxide (H_2O_2) on the colony. This indicates that the isolate no. 8 and standard bacterium, both are catalase positive.

Table 2: Sugar broth fermentation results

Sugars	Results
Glucose	+
Sucrose	-
Lactose	-
Maltose	-
Mannitol	-
Indole	No red ring

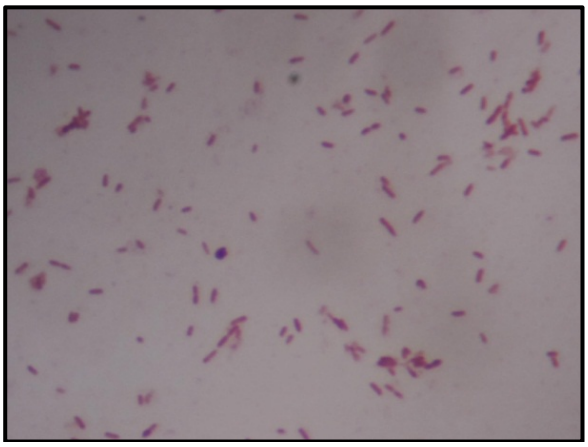

Figure 2: Gram negative short rods of isolate no. 8

DISCUSSION

Non gassy fermentation of glucose, nitrate reduction and catalase positive tests given by Gram negative short rods are significant for the confirmation of genus Pseudomonas. Zone of clearance in the tributyrin agar is a result of breakdown of tributyrin oil present in the medium. Rhodamine- B is added to the medium to form a good contrasting background. Breakdown of oil is possible only if there is a presence of Lipase enzyme in the medium.

CONCLUSION

The results confirmed the production of lipase by the isolate of *Pseudomonas sp.* from oil contaminated soil. Production and purification of extracellular lipase produced by this Pseudomonas isolate is possible. Hence it will be a promising future to produce lipase economically from soil isolate as compared to commercially available lipases which is expensive.

REFERENCES

1. Saeed HM, Zaghloul TI, Khalil AI, Abdelbaeth MT. Purification and characterization of two extracellular lipases from *Pseudomonas aeruginosa* Ps- x. Pol J Microbiol 2005; 54(3):233-240.
2. Sharma R, Chisti Y, Banerjee UC. Production, purification, characterization and applications of lipases. Biotechnology Advances 2001; 19:627-662.
3. Aravindan R, Anbumathi P, Viruthagiri T. Lipase applications in food industry. Indian J Biotechnol 2007; 6:141-158.
4. Sharma D, Sharma B, Shukla AK. Biotechnological Approach of Microbial Lipase: A Review. Biotechnology 2011; 10(1):23-40.
5. Kordel M, Hofmann B, Schomburg D, Schmid RD. Extracellular Lipase of *Pseudomonas* sp. Strain ATCC 21808: Purification, Characterization, Crystallization and

Preliminary X-Ray Diffraction Data. J Bacteriol 1991; 173(15):4836-4841.
6. Verma N, Thakur S, Bhatt AK. Microbial Lipases: Industrial Applications and Properties (A Review). Int Res J of Biol Sci 2012; 1(8):88-92.
7. Santhini K, Myla J, Sajani S, Usharani G. Screening of *Micrococcus* Sp. from Oil Contaminated Soil with Reference to Bioremediation. Botany Research International 2009; 2(4):248-252.
8. Sirisha E, Rajasekar N, Narasu ML. Isolation and Optimization of Lipase Producing Bacteria from Oil Contaminated Soils. Adv Biol Res 2010; 4(5):249-252.
9. Mohan TS, Palavesam A, Immanvel G. Isolation and characterization of lipase-producing *Bacillus* strains from oil mill waste. Afr J Biotechnol 2008; 7(15):2728-2735.
10. Godkar PB, Godkar DP. Textbook of medical laboratory technology. 2nd ed. India: Bhalani Publishing House; 2004. p. 549, 564.

Cite this article as:

Amol A. Shirsath *et al*. Screening and isolation of lipase producing Pseudomonas sp. from oil contaminated soil. Int. Res. J. Pharm. 2019;10(3):178-180 http://dx.doi.org/10.7897/2230-8407.1003100

Source of support: Nil, Conflict of interest: None Declared