International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 1(K); January 2018; Page No. 9492-9496
DOI: http://dx.doi.org/10.24327/ijcar.2018.9496.1571

Research Article

CHARACTERIZATION AND PRODUCTION OF FUNGAL LIPASES ISOLATED FROM DIESEL OIL CONTAMINATED SOIL

Mahima Golani[1, 2], Krishnan Hajela[1] and Govind Prakash Pandey[1]

[1]School of Life Sciences, Devi Ahilya University, Indore (M.P.), India
[2]PMB Gujarati Science College, Indore (M.P.), India

A R T I C L E I N F O

Article History:
Received 16th October, 2017
Received in revised form 10th November, 2017
Accepted 26th December, 2017
Published online 28th January, 2018

Key words:

Lipases, *Penicillium* sp., production, bioremediation, diesel oil contaminated soil

A B S T R A C T

28 lipolytic fungal isolates were screened by enrichment culture technique from diesel oil contaminated soil on tributyrin enriched sabouraud agar medium plates. Out of 28 fungi, isolate 4b which was identified as *Penicillium* sp. a promising strain for lipase production showed maximum zone of hydrolysis around colony with maximum lipase activity (2.5). The specific lipase activity of *Penicillium* sp. was found to be 2.08 µM/min/mg. Results indicates that indigenous soil microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation.

INTRODUCTION

Lipases (triacylglycerol acyl-hydrolases EC3.1.1.3) catalyze the hydrolysis of the ester bonds of insoluble substrates in water at the substrate water interface (Sharma *et al*. 2001). The diverse industrial applications of lipases developed interest in isolation of new lipases from novel sources (Marques *et al*. 2014). Oil and dairy products spilled soil harbors fungal species which have the potential to secrete lipases to degrade fats and oils (Niyonzima *et al*. 2014). The fungi have played a vital role in bioremediation and well documented (Gopinath *et al*. 2005). Major genera of filamentous fungi include *Rhizopus, Aspergillus, Penicillium, Mucor, Ashbya, Geotrichum, Beauveria, Humicola, Rhizomucor, Fusarium, Acremonium, Alternaria, Eurotrium* and *Ophiostoma* (Singh *et al*. 2012).

Bioremediation of waste disposal is a new way of approach in lipase biotechnology. Fungal flora can be applied to degrade oil spills in the coastal environment which may increase ecorestoration (Gopinath *et al*. 1998). Species belonging to the genera *Trichoderma*, *Fusarium*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Mortierella*, *Beauveria,* and *Engyodontium*are some examples of the fungi that have recently been described as tolerant to a variety of pollutants and indicated as potential bioremediation agents in soil (Islam *et al*. 2015). Other genera like *Staphylococcus, Streptococcus, Acinetobacter, Burkholderia, Arthrobacter, Achromobacter, Alcaligenes, Chromobacterium* and *Streptomyces* (Abramic *et al*. 1999; Riaz *et al*. 2010; Sangeetha *et al*. 2011) have been studied as lipase producers. Cleaning up of petroleum hydrocarbons in the subsurface environment is a real world problem. Laboratory scale experiments (Bartha biometer flasks) were used to evaluate the biodegradation of the diesel oil. Enhancement of biodegradation was carried out through bio stimulation (addition of nitrogen and phosphorus solutions or Tween 80 surfactant) and bio augmentation (bacterial consortium isolated from a land farming system) (Mariano *et al*. 2007). Indigenous and exogenous soil microorganisms were used for the assessment of the enzyme lipase as indicator of microbial degradation of crude oil (Ugochukwu *et al*.2008).

The main objective of the present study was to isolate and identify indigenous fungal flora from diesel oil contaminated soil and evaluate the biodegradation efficiency of potential isolates by lipase enzyme production.

MATERIALS AND METHODS

Collection of Soil Sample

The soil investigated was collected from the top 20 cm of the soil surface at the Botanical garden of PMB Gujarati Science College, 1-Nasia road, Indore, (M.P.). The main features of soil were analyzed.

Design of Bioremediation Experiments

Eight pans were prepared, each containing 1 kg of sieved soil at 45% of its water holding capacity (WHC); each pan, except treatment S, was spiked at 10g of diesel oil/kg soil and treated as follows:

Corresponding author:* **MahimaGolani
School of Life Sciences, Devi Ahilya University, Indore (M.P.), India

(S) Uncontaminated control soil; (CS) contaminated soil; (CSF) contaminated soil + N-P-K fertilizer; (CSC) contaminated soil + compost; (CSI) contaminated soil + Inoculum; (CSFI) contaminated soil + N-P-K fertilizer + Inoculum; (CSCI) contaminated soil + compost + Inoculum; (SCSCI) sterile (oil) contaminated soil + compost + Inoculum. CSI, CSFI, CSCI, SCSCI pots were inoculated with 10 ml inoculum of *Staphylococcus argenteus* MG2 (O1A) a noveland potentiallipase producing bacterium in order to evaluate hydrocarbon degradation by the indigenous soil microorganisms along with oil degrading bacteria and biological fertilizers.

The pans were incubated in the dark at 25ºC for 50 days. The water content of the pans was adjusted and maintained at 50% of the WHC (Water Holding Capacity) during the whole incubation period. Pan contents were thoroughly mixed on every second day for assurance of sufficient aerobic condition.

At the end of 10, 20, 35, 50 days, chemical and biological analyses were performed by sampling 3 soil sub-samples from each pan (Riffaldi *et al*., 2006).

Chemical Analysis

Soil pH - 10 gm of soil sample was collected from each experimental pot after completion of their treatment period and was mixed with sterile distilled water in a beaker stirred and allowed to stand for 30 mins; the soil pH then was determined with a glass electrode of digital pH meter.

Standard Plate Count (SPC) of Oil Degrading Microorganisms

The total microbial count and oil-degrading microorganisms from each experimental pot soil after completion of their treatment of time period interval was determined by the standard plate count method for viable cells as described (Margesin and Schinner 1999a), using tributyrin agar medium plates containing 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 1% (v/v) tributyrin, 2% (w/v) agar, pH 7.0 (complete medium). 10g of the diesel oil contaminated soil samples along with control was weighed and then added in 100 ml sterile distilled water. Serial dilutions of the soil samples were prepared up to 10^{-6}. Hundred microliter of appropriate dilution was plated in above mentioned medium plates by spread plate method. Enumerations were made with triplicates. Colony forming units (cfu) were counted after 7 days at 28ºC. Some oil degrading fungi producing maximum zone of hydrolysis around the colony were selected for further study to check their efficiency during this soil bioremediation experiment.

Isolation and identification of oil degrading Fungi

Some efficient and isolated oil degrading fungi from 1% diesel oil agar plates of soil bioremediation experiment were isolated on Tributyrin enriched Sabouraud agar medium plates and incubated at 28˚C for 7 days. The zone of clearance around the isolated fungal colony was observed due to hydrolysis of tributyrin by lipase. Some other samples like vegetable oil contaminated soil of Navlakha region and oil contaminated soil of Ruchi Soya Industry were also analysed for isolation of fungi. Pure cultures of the isolate were maintained on MRBA agar medium slants at refrigerated 4^{o} C temperature and were sub cultured every month.

Characterization and Identification of Selected Fungal Isolate

The fungal isolates found to produce maximum zone of hydrolysis (Table 2) around the colony was selected and was studied for its morphological and colonial characteristics.

Morphological Characteristics

The size, shape and arrangement of the fungal cells were studied by wet mount method using lacto phenol cotton blue stain.

Lipase Production by Fungal Isolate 4b for Lipase Activity

For lipase production of fungal isolate 4b, 500-ml Erlenmeyer flasks each containing 100 ml of tributyrinen riched sabouraud broth medium was inoculated with 1% of inoculum and incubated at 28ºC in orbital shaker at ar otary speed of 150rpm for 4 days. The crude broth was harvested, aseptically by high speed cooling centrifugation at 10,000g for 30min at 4ºC. The supernatant collected was used as crude enzyme solution and was assayed for enzyme activity by titrimetric method.

RESULTS

Collection of Soil Sample

The soil investigated was collected from the top 20 cm of the soil surface at the Botanical garden of PMB Gujarati Science College, 1- Nasia road, Indore (M.P.). The main features of experimental soil analyzed were, Sand- 69.0 (%), Slit- 17.2 (%), Clay- 13.8 (%), WHC- 45.0 (%), $CaCO_3$- 17.6 (%), Organic- 1.38 (%), pH- 7.5.

Design of Bioremediation Experiments

(S), (CS), (CSF), (CSC) pots were not inoculated with any culture while CSI, CSFI, CSCI, SCSCI pots were inoculated with *Staphylococcus argenteus* MG2 (O1A) a novel and potential lipase producing bacterium in order to evaluate hydrocarbon degradation by the indigenous soil microorganisms along with oil degrading bacteria and biological fertilizers.

The 16S rDNA nucleotide sequence of the isolate *Staphylococcus argenteus* MG2 determined in this study has been deposited in the (NCBI, US) Gene Bank database under the accession number KY082046. The culture identified as *Staphylococcus argenteus* MG2 is deposited in Microbial Type Culture Collection Center and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India; under the accession number MTCC 12820. After fifty days of bioremediation treatment of soil contaminated with diesel oil was used to check that which quality of soil is appropriate for the growth of fungi.

Chemical Analysis

Soil pH-The soil pH remained in the neutral to slightly alkaline range (pH 7-8) during the whole incubation period independent of fertilizer.

SPC of Oil Degrading and Total Microbial Count on Tributyrin Medium Agar plate

Oil Degrading and Total Microbial Count on tributyrin medium agar plate during bioremediation treatment of soil contaminated with diesel oil were observed. Tributyrin agar

medium is a complete medium which supports the growth of various kinds of microorganisms therefore not only oil degrading microbes were growing but various types of bacteria, fungi and actinomycetes also.

Among the bacterial colonies *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., and various types of yellow, orange, mucoid and black pigmented colonies were identified. Various fungi like *Penicillium* sp., *Aspergillus* sp., Yeast and many other types of fungi were able to degrade oil present in the soil.The number of oil degrading and total microbial count on tributyrin medium agar plate was found to be higher in SCSCI soil sample.

Screening of Lipase Producing Fungi

Enrichment, isolation and screening of lipolytic fungi

28 Lipolytic fungal isolates were screened by enrichment culture technique from soil bioremediation experiment samples and some other samples like vegetable oil contaminated soil of Navlakha region and soy oil contaminated soil of Ruchi Soya Industry on tributyrin enriched sabouraud agar medium plates and zone of clearance around the isolated fungal colony was observed due to hydrolysis of tributyrin by lipase. Five different types like orange, grey, green, and white colored fungi were isolated from vegetable oil contaminated soil of Navlakha region and 3 different kinds of fungi like grey, olive green and jet black colonies were isolated from soy oil contaminated soil of Ruchi Soya Industry but they were not degrading oil well. Cultural characteristics and lipase activity of 20 fungi isolated from soil bioremediation experiment samples are reported in Table - 1 and 2.

Pure cultures of the isolate were maintained on MRBA medium agar slants at refrigerated 4^{o} C temperature and were sub cultured every month. Out of 28 fungi, 03 isolates were found to be growing well. Among the 03 isolates, 4b (Figure 1) (Table 2) showed maximum zone of hydrolysis around colony with maximum lipase activity (2.5) which was selected for further studies.

Figure 1 Growth of fungal isolate 4b

Characterization of Selected Fungal Isolate

Morphological and cultural characterization of selected isolate

The morphological and cultural studies of selected isolate 4b, was performed which depicts the cultural characters of isolate 4b (Table 1). The isolate 4b was found to be like penicillium sp. (CSC) which shows palm like arrangement on staining with lacto phenol cotton blue (Figure 2).

Table 1 Cultural characterstics of lipolytic fungal isolate

S.No.	Designation of Isolates	Source of sample	Colony morphology
1	1a	Soil sample of (S) uncontaminated soil	Cottony and slight fluffy colonies with black spores on aging
2	1b	Soil sample of S (uncontaminated soil)	Olive greenish wooly, elevated, round and even colonies
3	2a	Soil sample of (CS) contaminated soil	Cottony, fluffy colonies with dirty spores on aging
4	2b	Soil sample of (CS) contaminated soil	Cottony and slight fluffy colonies with black spores on aging
5	4a	Soil sample of (CSC) contaminated soil + compost	Flat greenish colonies and in center dark black spores on thread like mycelium
6	4b	Soil sample of (CSC) contaminated soil + compost	White cottony fluffy folded and elevated colonies with green spores on aging
7	4c	Soil sample of (CSC) contaminated soil + compost	Greenish cottony fluffy colonies
8	4d	Soil sample of (CSC) contaminated soil + compost	Cottony fluffy colonies with yellow spores on aging
9	5a	Soil sample of (CSI) contaminated soil + Inoculum	Dull greenish powdery round colonies
10	5b	Soil sample of (CSI) contaminated soil + Inoculum	White cottony colonies with green spores on aging
11	5c	Soil sample of (CSI) contaminated soil + Inoculum	Flat colonies with green spores on aging
12	6a	Soil sample of (CSFI) contaminated soil + N-P-K fertilizer + Inoculum	Peach colored cottony icy fluffy, wooly and elevated colonies with thread like hyphae
13	6b	Soil sample of (CSFI) contaminated soil + N-P-K fertilizer + Inoculum	White cottony raised colonies with green spores on aging
14	6c	Soil sample of (CSFI) contaminated soil + N-P-K fertilizer + Inoculum	Dull green fluffy concentric colonies with white spores on aging
15	7a	Soil sample of (CSCI) contaminated soil + compost + Inoculum	Creamish cottony colonies with dull green powdery spores on aging
16	7b	Soil sample of (CSCI) contaminated soil + compost + Inoculum	Olive green cottony fluffy colonies
17	7c	Soil sample of (CSCI) contaminated soil + compost + Inoculum	White cottony raised colonies with creamish spores on aging
18	7d	Soil sample of (CSCI) contaminated soil + compost + Inoculum	Dark green and slightly elevated colonies with powdery green spores
19	8a	Soil sample of (SCSCI) sterile (oil) contaminated soil + compost + Inoculum	White cottony large flat colonies
20	8b	Soil sample of (SCSCI) sterile (oil) contaminated soil + compost + Inoculum	Medium size green colonies

Table 2 Comparative analysis of lipase activity by fungal isolates

S. No.	Designation of Isolates	Zone of lipid hydrolysis (mm)	Growth zone (mm)	Lipolytic activity *
1	1a	34	25	1.36
2	1b	40	23	1.73
3	2a	40	27	1.48
4	2b	72	65	1.1
5	4a	33	25	1.32
6	4b	20	8	2.5*
7	4c	41	34	1.2
8	4d	40	33	1.21
9	5a	33	17	1.94
10	5b	33	20	1.65
11	5c	18	13	1.38
12	6a	40	19	2.1
13	6b	48	23	2.08
14	6c	30	24	1.25
15	7a	23	10	2.3
16	7b	35	30	1.16
17	7c	35	30	1.16
18	7d	25	13	1.92
19	8a	35	25	1.4
20	8b	28	21	1.33

* Lipolytic activity = Hydrolysis zone (mm): growth zone (mm)

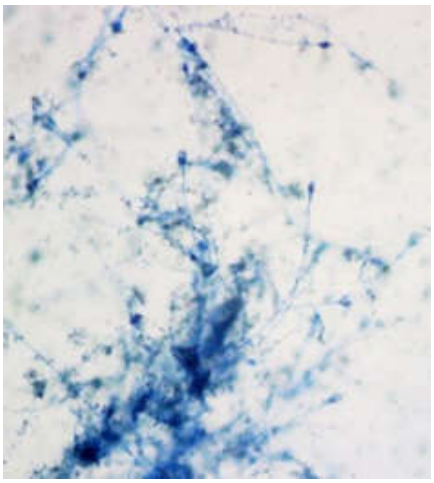

Figure 2 Morphological Characters of fungal isolate 4b

Lipase Production by Fungal Isolate 4b for Lipase Activity

The specific lipase activity of fungal isolate 4b in tributyrinen riched sabouraud broth medium was found to be 2.08 µM/min/mg. Results indicates that the specific lipase activity was found to be slightly less than the *Staphylococcus argenteus* MG2 bacterium (2.30 µM/min/mg).

DISCUSSION

The specific lipase activity of potential *Penicillium* sp. was 2.08 µM/min/mg. This efficient lipase producing *Penicillium* sp. was isolated from Soil sample of (CSC) contaminated soil+ compost. The compost may have facilitated the growth of fungi because it plays an important role in supplementing continuously nutrient and carbon sources. Jesus *et al*. 1999 obtained 1.1 U/mg specific activity of lipase by *Penicillium* restricum. Similar lipase from *Penicillium sp.* was optimized by Wolski *et al*., 2008. Balaji *et al*. 2014 and wadia *et al*. 2017 also isolated *Penicillium* sp. from oil contaminated soil.

CONCLUSION

This study concludes that fungi are potent lipase producers.It was observed during the whole experiment that the microbial growth in SCSCI appeared in 2 days while other soil samples required 4-5 days. Microbial growth was found to be good in SCSCI which was followed by CSCI and CSFI respectively. Fungus was screened from all types of soil samples Thus Bio stimulation resulted in significantly increased counts of oil degraders. From the results obtained above, it can be understood that bio stimulation can effectively be used to combat pollution. The findings of other researchers also support the fact that bio stimulation could indeed be a solution for degrading environmental pollutants (Miller, 2010). The results also prove that bio stimulation is an effective method of reducing environmental pollution. Thus, the present study concludes that microbial degradation can be regarded as a key component in the bioremediation of petroleum hydrocarbon.

Acknowledgement

The financial assistance provided by University Grant Commission Bhopal (MS-29/103042/12-13/CRO) and facilities provided by the Management and Principal of PMB Gujarati Science College and School of Life Sciences, DAVV, Indore is great fully acknowledged.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Abramic M, Les I, Korica T, Vitale L, Saenger W, Pigac J (1999). Purification and properties of extracellular lipase from *Streptomyces rimosus*. *Enzyme Microbial Technol*., 25: 522-529.
2. Balaji V. Arulazhagan P and Ebenezer P (2014). Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. *J. of Env. Bio*. Vol. 35, 521-529.
3. Gopinath S, Hilda A, Ramesh VM (1998). Detection of biodegradability of oils and related substances. *J Environ Biol*. 19(2): 157-165.
4. Gopinath SCB, Anbu P, Hilda A (2005). Extracellular enzymatic activity in fungi isolated from oil rich environments. *Mycoscience*. 46(2): 119-126.
5. Islam R and Datta B (2015). Fungal diversity and its potential in environmental cleanup. *Int J Res*. 2: 815-825.
6. Jesus MFCP, Branco RN, Santanna GLJR, Freire DMG, Silva JGJR (1999). *Penicilliumrestricum* lipases: A comparative study and characterization of enzymes with different degrees of purity. *Braz. J. Chem. Eng*. Vol. 16 n.2.
7. Margesin R, Schinner F (1999a*). World J. Microbiol. Biotechnol.* 15: in press.
8. Mariano PA, Arruda Geraldes Kataoka PA, Angelis FD, Bonotto MD (2007). Laboratory study on the bioremediation of diesel oil contaminated soil from a petrol station. *Braz. J. Microbiol*. Vol.38 no.2.

9. Marques TA, Baldo C, Borsato D, Buzato JB, Celligo MAPC (2014). Production and partial characterization of a thermostable, alkaline and organic solvent tolerant lipase from Trichoderma atroviride 676. *Int J Sci Technol Res*. 3(5): 77-83.
10. Miller H (2010). *Basic Biotechnology*. 6, 7-12.
11. Niyonzima FN, More S (2014). Biochemical properties of the alkaline lipase of Bacillus flexus XJU-1 and its detergent compatibility. *Biologia*. 69(9): 1108-1117.
12. Riaz M, Shah AA, Hameed A, Hasan F (2010). Characterization of lipase produced by *Bacillus* sp. FH5 in immobilized and free state. *Ann. Microbiol*., 60: 169-175.
13. Riffaldi R, Levi-Minzi R, Cardelli R, Palumbo S and Saviozzi A (2006). Soil Biological Activities in Monitoring the Bioremediation of Diesel Oil Contaminated Soil. *Journal Water, Air, and Soil Pollution.* 170: 3-15.
14. Sangeetha R, Geetha A, Arulpandi I (2010). Concomitant production, partial purification and characterization of a serine protease and a proteolysis-resistant metallolipase from *Bacillus pumilus* SG2. Z. Naturforsch, 65: 61-65.
15. Sharma R, Chisti Y, Banerjee UC (2001). Production, purification, characterization and applications of lipases. *Biotechnol Adv*. 19(8): 627-662.
16. Singh AK, Mukhopadhyay M (2012). Overview of Fungal Lipase: A Review. *Appl Biochem Biotechnol.* 166(2): 486-520.
17. Ugochukwu CK, Agha CN and Ogbulie NJ (2008). Lipase activities of microbial isolates from soil contaminated with crude oil after bioremediation. *African J. of Biotech*. Vol. 7(16), pp. 2881-2884.
18. Wadia T and Jain KS (2017). Isolation, screening and identification of lipase producing fungi from oil contaminated soil from Shani Mandir Ujjain. *Int. J. Curr. Microbio. App. Sci.* 6(7): 1872-1878.
19. Wolski E, Menusi E, Mazutti M, Toniazzo G, Rigo E, Cansian RL, Mossi A, Oliveira JV, Luccio MD, Oliveira JV, Luccio MD, Oliveira D, Treichel H (2008). Response surface methodology for optimization of lipase production by an immobilized newly isolated Penicillium sp. *Ind Eng Chem Res.* 47: 9651-9657.

How to cite this article:

Mahima Golani *et al* (2018) 'Characterization and Production of Fungal lipases isolated From Diesel Oil Contaminated Soil', *International Journal of Current Advanced Research,* 07(1), pp. 9492-9496.
DOI: http://dx.doi.org/10.24327/ijcar.2018.9496.1571

****