International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 06
May 2017

Isolation and screening of lipase producing fungi from soil

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Abstract:

Lipases are glycerol ester hydrolases that catalyze the hydrolytic cleavage of ester bonds of water insoluble triglycerides to free fatty acids and glycerol. Soil is an excellent habitat of variety of enzyme producing microbes. Hence, soil samples were collected from 4 different regions and used for isolation of fungi. Fungi with different colony characteristics (zonation, sporulation, colouration, texture, shape and size) were appeared on potato dextrose agar plates. Based on difference in their appearance, a total of 27 fungi were selected for screening on tributyrin agar (TBA) plates for identification of lipase producers. Among these, 6 fungal isolates demonstrated halo zone around their colonies on TBA plates. 19 isolates did not show any halo zone around their colonies and remaining 2 isolates did not grow on TBA plates.

Key words :Lipases, triglycerides, fungi, soil, tributyrin.

1. Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis of water insoluble ester molecules (long carbon chain fatty acids containing triacylglycerol) into monoacylglycerol, diacylglycerol, glycerol and free fatty acids. Lipases are found in plants, animals and microorganisms, but only microbial lipases are industrially significant. The demand of microbial lipases is higher because of their specificity of reaction, stereo specificity and fewer energy uses than traditional techniques [1]. Numerous microbes such as fungi, bacteria and yeast are well-known to secret lipases. Among these, the filamentous fungi are well known as perfect source of lipases since they produce extracellular enzyme [2]. Some of the industrially significant lipolytic fungi are of the genera *Penicillium*, *Geotrichum*, *Aspergillus*, *Candida*, *Rhizomucor* and *Rhizopus* [3]. The demand of industries for novel sources of lipases with dissimilar enzymatic features stimulates the isolation and identification of novel strains of lipase producing microbes [4].

Lipolytic microorganisms have been establish in different environment such as effluent from pulp and paper industry, edible oil processing factories, dairy industries and soil contaminated with edible oil, petrol and diesel oil [5]. The soil has a huge diversity of microbes that can be isolated and assessed for their capabilities as various enzyme production [6]. The isolation and identification of microbes can lead to the secretion of lipases with stability to broad range of pH and temperatures, specificity to some fatty acids, and enantioselectivity [7].

Numerous techniques can be utilized for screening of lipolytic microorganism based on the detection of the occurrence of extracellular lipases. The utilization of a solid agar medium containing inducer substrates such as edible oils, triglycerides (triolein, tributyrin), Tween-20, Tween-80, and various dyes [6, 8] has been broadly explained in the literature.

Lipases are being utilized in various industries such as detergent formulation, textiles, oleochemistry, paper, organic synthesis, dairy, nutrition and tea [1]. Consequently, the significance of fungal lipase research has risen enormously [9]. In view of this background we have collected soil samples from different areas and used them for isolation of fungi followed by their screening on tributyrin agar medium for identification of lipase producers.

2. Materials and Methods

2.1 Collection of soil samples

Soil samples were collected using sterile spatula from 4 diverse areas (mustard crop field, wheat crop field, petrol pump of Newai Town and medicinal garden of Banasthali University) and transferred in sterile Petri plates. Petri plates were labeled with the site of collection, brought to laboratory and stored in refrigerator till use.

2.2 Isolation of soil fungi

Fungi were obtained by serial dilution agar plate method as explained earlier by Waksman [10]. One gram of each of the soil sample was separately added in test tube containing 9 ml of sterile distilled water followed by vortexing for 10 minutes to make soil suspension of 10^{-1} dilution. One ml of soil suspension from 10^{-1} dilution was transferred in another test tube containing 9 ml of sterile distilled water to prepare 10^{-2} dilution. This step (withdrawal and transfer of soil suspension) was repeated until the preparation of 10^{-6} dilution. 0.1 ml of each of the soil dilution (10^{-1} to 10^{-6}) was aseptically inoculated and uniformly spreaded in sterile Petri plate containing the potato dextrose agar (PDA) medium [11]. Streptomycin antibiotic was added in PDA medium just before pouring of PDA medium into Petri plates to avoid growth of bacteria in PDA plates. The inoculated PDA plates were kept at 28ºC for 5 days in an incubator. Different fungi appeared on PDA plates were further purified by point inoculation in PDA plates followed by incubation.

2.3 Screening on tributyrin agar medium

Fungal isolates were assessed for detection of lipase production on tributyrin agar (TBA) medium. The composition of TBA medium (g l^{-1}) is as follows: peptone, 5; yeast extract, 3; agar, 15; tributyrin (glycerol tributyrate), 10 and pH was adjusted to 7.5. The isolates were individually point inoculated on TBA plates and kept at 28˚C for 6-8 days. Inoculated TBA plates were observed for halo zone around fungal colonies [2].

3. Results and Discussion

3.1 Isolation of soil fungi

The results of isolation are presented in Table 1 and Fig. 1 to 4. Diverse fungi with different colony morphology and characteristics (texture, sporulation, zonation, shape, size, surface and reverse colouration) were appeared on PDA plates from different dilutions of soil samples (Figure 1 to 4). The texture of the isolated fungi was velvety, floccose and powdery. The zonation of isolated fungi was slightly radially furrowed to heavily furrowed on reverse side. Poor, moderate and heavy sporulation pattern was observed. The colour of isolated fungi was black, white, brown, orange, creamish, chocolate and green. Some of the fungi were growing all around Petri plate. Most of the fungi were circular in shape and remaining fungi were irregular in shape.

Table 1 represents that maximum load of fungi were obtained from soil sample contaminated with petroleum oil whereas least number of fungi were obtained from the soil sample of wheat crop field.

3.2 Screening

Based on the difference in colony morphology and characteristics different fungal isolates were used for screening in TBA medium. Lipase producing fungi were identified by the appearance of zone of hydrolysis (clear zone/halozone) around their colonies (Figure 5). Clear zone was developed due to hydrolysis of tributyrin by extracellular lipase into components, therefore opacity of the medium around fungal colony was not retained. Presence of opacity around fungal colony indicated that fungal colony did not produce extracellular lipase in its vicinity, therefore tributyrin was not degraded, hence opacity of the medium was retained. Very few isolates were unable to grow on TBA medium.

Several researchers [13, 14] have been utilized tributyrin agar plates for identification of lipase producing fungi from oily soil samples. Veerapagu *et al.* [15] utilized tributyrin agar medium and Rhodamine olive oil agar (ROA) medium for screening of lipase producers. Among the 200 isolates, 32 isolates demonstrated high activity on TBA plates and 12 isolates exhibited lipolytic activity on ROA plates.

Vargas *et al.* [4] investigated the lipolytic activities of 150 isolated strains from samples of Lake Bogoria (Kenya). Among all, 15 isolates were selected based on their lipolytic potential for identification by 16-S-rRNA sequencing. Colen *et al.* [3] reported isolation of 59 fungal strains from Brazilian savanna soil. All isolates were tested for identification of lipolytic strains in agar plate containing olive oil and bile salts. Among all, 21 fungal strains were chosen by the appearance of halo zone around their colonies. Lipolytic potential of 21 strains were further confirmed by secondary screening in submerged fermentation as a result 11 strains were found god producer of lipase.

Soni and Sharma [12] reported isolation of mycoflora by serial dilution agar plate technique from soil samples of different regions of Bhopal. Isolated fungi were identified as *Aspergillus fumigates*, *Aspergillus niger*, *Fusarium*, *Trichoderma* and *Curvularia* based on microscopic characteristics and Petri plate culture. Total 14 species were obtained from 20 soil samples. Among all the species, *Aspergillus niger* was dominant.

Ko *et al.* [6] used agar medium containing 0.1% sunflower oil and 0.01% Tween 80 for screening of lipolytic microbes. Lipolytic colonies of microbes were surrounded by clear zone of hydrolysis of oil. The major population of lipolytic microbes in an agricultural soil was actinomycetes followed by bacteria and fungi. Generally, comparatively little fractions of soil bacteria were found lipase producers, and lipase production was more frequent among soil actinomycetes and fungi.

Pandey *et al.* [2] reported isolation of 80 fungal species from soil samples followed by their screening on tributyrin agar medium for identification of lipolytic fungi. Among these, only 6 fungal strains were demonstrated zone of hydrolysis on tributyrin agar plates, indicated production of extracellular lipase. Among 6 fungi, 4 were identified as *Penicillium* genus, remaining 2 were identified as *Aspergillus* and *Alternaria* genus.

4. Conclusion

Soil samples collected from 4 diverse regions of Newai Town were serially diluted up to 10^{-6} dilution and utilized for isolation of fungi on PDA plates. Several fungi were isolated on PDA plates. Fungi exhibited different colony morphology and characteristics. A total of 27 fungi were selected for primary screening on TBA plates and among these, 6 isolates were identified as lipase producers based on the determination of zone of hydrolysis around their colonies. Lipolytic potential of these 6 isolates can be further confirmed by secondary screening in submerged fermentation.

5. References

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Table 1. Total number of fungal colonies appeared on PDA plates from four different soil samples.

Dilutions	Total number of fungal colonies on PDA plates			
	Mustard crop field	Medicinal garden field	Wheat crop field	Petrol contaminated
10^{-1}	TNTC	TNTC	TNTC	26
10^{-2}	11	21	TNTC	12
10^{-3}	5	13	9	11
10^{-4}	2	5	6	5
10^{-5}	2	2	3	2
10^{-6}	2	3	1	8

TNTC: Too numerous to count.

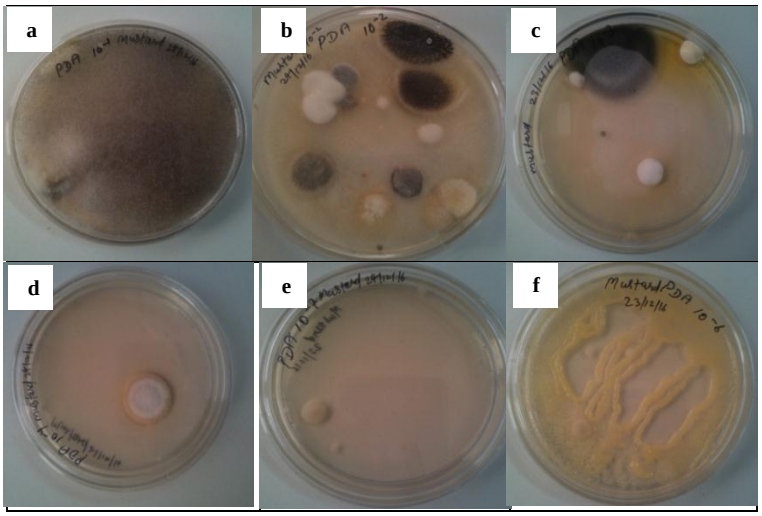

Figure 1. PDA plates showing different fungal colonies in different dilutions of soil sample (mustard crop field) **(a):** Dilution 10^{-1}; **(b):** Dilution 10^{-2}; **(c):** Dilution 10^{-3}; **(d):** Dilution 10^{-4}; **(e):** Dilution 10^{-5} **(f):** Dilution 10^{-6}.

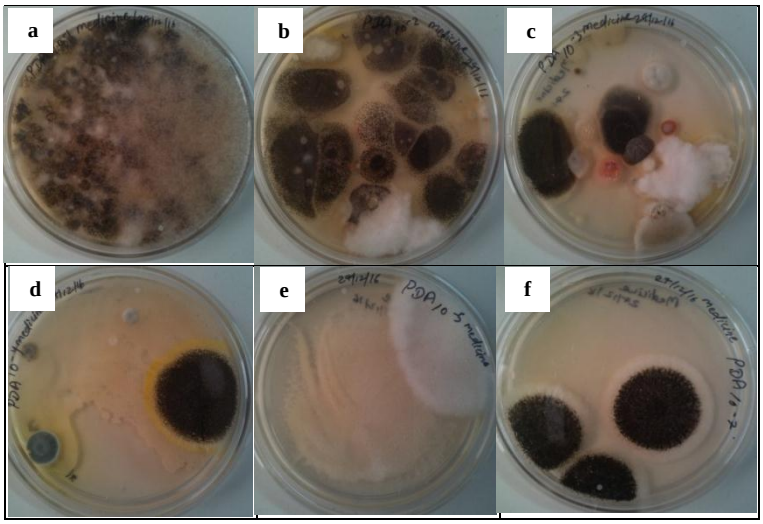

Figure 2. PDA plates showing different fungal colonies in different dilutions of soil sample (medicinal garden) **(a):** Dilution 10^{-1}; **(b):** Dilution 10^{-2}; **(c):** Dilution 10^{-3}; **(d):** Dilution 10^{-4}; **(e):** Dilution 10^{-5} **(f):** Dilution 10^{-6}.

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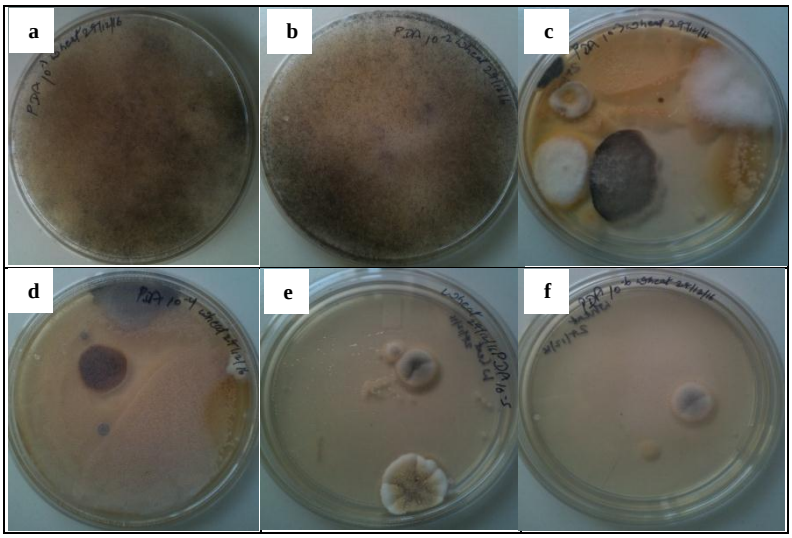

Figure 3. PDA plates showing different fungal colonies in different dilutions of soil sample (wheat crop field) **(a):** Dilution 10^{-1}; **(b):** Dilution 10^{-2}; **(c):** Dilution 10^{-3}; **(d):** Dilution 10^{-4}; **(e):** Dilution 10^{-5} **(f):** Dilution 10^{-6}.

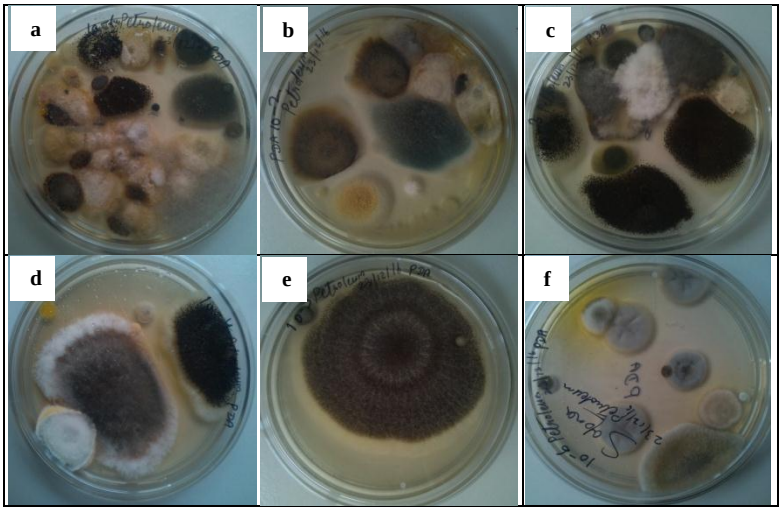

Figure 4. PDA plates showing different fungal colonies in different dilutions of soil sample (petrol contaminated) **(a):** Dilution 10^{-1}; **(b):** Dilution 10^{-2}; **(c):** Dilution 10^{-3}; **(d):** Dilution 10^{-4}; **(e):** Dilution 10^{-5} **(f):** Dilution 10^{-6}.

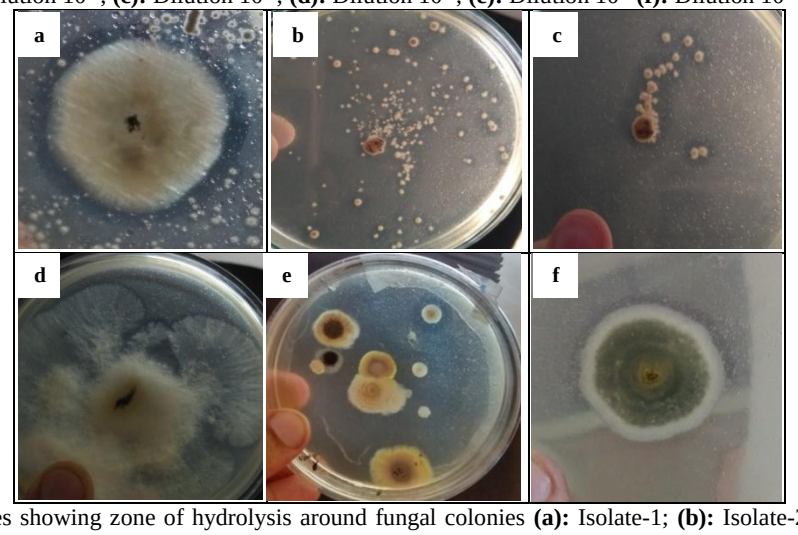

Figure 5. TBA plates showing zone of hydrolysis around fungal colonies **(a):** Isolate-1; **(b):** Isolate-2; **(c):** Isolate-3; **(d):** Isolate-4; **(e):** Isolate-5 and **(f):** Isolate-6.