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ISOLATION AND SCREENING OF LIPOLYTIC SOIL FUNGI

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ABSTRACT

Lipases are a bunch of enzymes that act on triacylgycerol and liberate fatty acids and alcohol. Microbial lipases are most significant extracellular substances and are important for scientific study because of their vital usage. In this context, the present study was conducted to isolate lipolytic fungi from the soil samples. A total of 20 fungal strains were selected for screening onto different media out of which 6 demonstrated zone of hydrolysis on tributyrin agar plates, 3 fungal strain exhibited orange colur fluorescent under UV (350 nm) on Rhodamine B agar plates, 3 lipolytic fungal strains were identified based on formation of precipitation around their colonies on Tween 80 agar plates and 8 isolates demonstrated yellow color on Phenol red agar plates around their colonies indicated secretion of extracellular lipase.

KEY WORDS

Lipase, fungi, tributyrin, soil.

INTRODUCTION

Human beings have been using enzymes for different purposes from ancient times. Presently, near about 4000 enzymes were reported and out of them 200 enzymes are in industrial application. Majority of commercial enzymes are microbic in origin and hydrolytic in nature (Godfrey and West, 1996; Wilke, 1999).

Lipases (triacylgycerol acylhydrolase, EC 3.1.1.3) are a cluster of enzymes that act on triacylgycerol and liberate fatty acids and alcohol (Abrunhosa et al. 2013). In non-aqueous phase this reaction is reversible (Tembhurkar et al. 2012).

Lipases of microbial origin that are engaged in the dairy, detergent, cosmetic, tanning, and food industries are frequently engaging. In view of their recent and potential applications, lipases are thought to be a promising category of commercial enzymes. Among these applications, the development of producing processes with microbic catalysts is one among the foremost vital area of analysis as a result of enzyme catalysed reactions are extremely selective and efficient, are less polluting, and fewer energy and usually need gentle conditions, that ends up in lowering the rate of production.

Lipases are reported to be produced by many species of animals, plants, bacteria, yeasts and fungi. Among all these, perfect source of extracellular lipase production are filamentous fungi. Different sources have been reported for the production of lipolytic fungi such as dairy industry, mustard field, edible oil processing industry and soil contaminated with oil (Sztajer et al. 1998).

Because of wide substrate specificity, consistency and selectivity lipases have gained unique industrial concern (Dutra et al. 2008; Griebeler et al. 2011). Microbic origin

lipases are steadier then animal and plant enzymes hence, production of lipase producing microbes is more easier and safer (Hasan et al. 2006). By keeping immense applications of lipases in mind, soil samples were collected and used for screening of lipase producing fungi.

Materials and methods

Collection of soil samples

The soil samples were collected from 6 different sites. Soil sample-1 was collected from Mustard oil processing industry, sample-2 was collected from dairy industry, sample-3 was collected from petrol pump, sample-4 was collected from motor garage, sample-5 was collected from agricultural field and sample-6 was collected from comb waste. Sterile spatula was used for the collection of soil samples in a sterile plastic bag and stored in refrigerator at 4℃.

Isolation of fungi from soil samples

Several dilution tubes were prepared containing 9 ml of sterile distilled water and labeled as 10^{-1} to 10^{-8}. One gram of each soil sample was added into sterilized glass test tube containing 9 ml of sterilized distilled water and 1 ml of mixture in first was transferred to second test tube containing 9 ml of sterilized distilled water and same process was done continuously until the last eighth test tube.

33.33 µl (0.3 mg/ml) of streptomycin from stock was added into PDA media after autoclaving to avoid bacterial growth and PDA media was poured into sterilized disposable petri plates. 0.1 ml of each dilution was aseptically spreaded onto the PDA plates by using a glass spreader. PDA plates were incubated at 28 ℃ for 5-7 days in inverting condition. Different fungal colonies which were appeared on plates were counted and subjected to qualitative screening of lipase producers. These fungal colonies were assigned the name (Kumar et al. 2015).

Screening of purifies cultures

Screening of lipolytic fungi was performed on four different agar media.

a) Tributryrin agar medium (TBA)

TBA media was prepared by dissolving 4.6 g of TBA in 198 ml of distilled water, 2 ml of tributyrin was added and 20 µl of tween 20 added as an emulsifier into the media (Wadia and Jain, 2017).

b) Rhodamine B olive oil agar medium

Media was prepared by dissolving 0.8 g of nutrient broth, 0.4 g of NaCl, 1 g of agar in 100 ml of distilled water (pH 7.0). After autoclaving 1.5 ml of olive oil and 10-20 µl of tween 20 was added. Add 1 ml (1 mg/ ml) of Rhodamine B solution and vigorously shaked (Kumar et al. 2012).

c) Phenol red agar medium:

Media was prepared by dissolving 0.1g of phenol red, 0.1 g of $CaCl_2$, 0.2 g of agar and 1 ml of tributyrin in 100 ml of distilled water (pH= 7.0) (Narasimhan and Valentin, 2015).

d) Tween 80 hydrolysis:

1.5 g of peptone, 0.5 g of NaCl, 0.1 g of $CaCl_2$, 1.5 g of agar was dissolved in 100 ml of distilled water and autoclaved. After autoclaving 1 ml of Tween 80 was added into the media (Kumar et al. 2012).

Fungal isolates were inoculated and streaked onto the different media plates and plates were incubated at 28ºC for 4-5 days in inverted condition. After incubation plates were analysed.

Results and Discussion:

Isolation of fungi:

The results are represented in Table 1. Diverse fungal colonies were obtained with different morphology and characterstics (size, elevation, colour, opacity, surface, margin, sporulation, zonation) were appeared on PDA plates. The pigmentation of isolated fungal strains was black, white, creamish, green, pink, grey and buff. The surface of fungal colonies was smooth, glistening, rough, wrinkled and dull. Mostly fungi were round in shape and few were uneven in shape. Fungal colonies appeared with different opacity for example, opaque, transparent (clear), translucent (like looking through frosted glass). Table 1 represents maximum number of colonies in Mustard oil soil sample and minimum number of colonies in comb wastes sample.

Colen et al. (2009) isolated 59 lipase producing fungal strains from Brazillian savanna soil by serial dilution method. All isolates were tested for identification of lipolytic strains on agar plate containing olive oil and bile salts. Choudhary et al. (2017) investigated the 13 lipolytic strains from oil mill soil sample. Among these, selected positive lypolytic strains were quantitative screen by titrimetric method. Vargas et al. (2004) investigated the lipolytic activities of 150 isolated strains from samples of Lake Bogoria (Kenya). Among all, 15

fungal isolates were selected based on their lipolytic potential for identification by 16-S-rRNA sequencing.

Table 1. Fungal colonies appeared on PDA plates

Dilutions	Number of fungal colonies					
	Mustard oil industry (Soil sample 1)	Dairy industry (Soil sample 2)	Petrol pump (Soil sample 3)	Motor garage (Soil sample 4)	Agricultural field (Soil sample 5)	Comb wastes (Soil sample 6)
10^{-1}	TNTC	26	TNTC	TNTC	TNTC	29
10^{-2}	TNTC	19	28	15	24	13
10^{-3}	18	15	21	8	14	8
10^{-4}	13	9	18	5	7	5
10^{-5}	8	6	9	3	4	1
10^{-6}	5	2	4	2	2	-
10^{-7}	2	1	1	2	2	-

TNTC: Too numerous to count.

Primary screening of soil fungi:

Different fungal isolates were grown onto four different media plates. Twenty fungal isolates of soil samples were subjected to screening in four different media. Six isolates demonstrated the zone of hydrolysis in tributyrin agar medium, indicated production of extracellular lipase by these isolates (Figure 1). Five isolates did not show any zone of hydrolysis indicated that they were growing on media without using the lipidic substrate for their growth. Remaining five isolates were not growing on that media. Three lipolytic fungal strains were identified on Rhodamine B olive oil agar medium by the formation of orange fluorescent halos around fungal colonies visible under UV irradiation (Figure 2).

Eight isolates demonstrated yellow color on Phenol red agar medium around their colonies indicated secretion of extracellular lipase (Figure 3). Three isolates did not show any yellow color around their colonies indicated growth without using any lipidic substrate. Three lipolytic fungal strains were identified by showing precipitation around fungal colonies on Tween 80 agar media (Figure 2). In our study we found tributyrin agar medium was the best media for screening of lipolytic microorganisms.

Fungal isolates of petrol pump soil sample demonstrated more lipase activity than other soil samples used in the investigation. This indicates that this particular habitat is more optimum for occurrence of lipase producing fungi.

Similarly, tributyrin agar medium was also used for screening of lipolytic microbes by number of investigators. Wadia and Jain (2017) reported that five fungi demonstrated zone of lipolysis out of a total of 15 tested fungi. *Aspergillus flavus*, *Trichoderma sp*., *Penicillium sp*., *Alternaria sp*. and *Aspergillus niger* were identified according to colony microscopic and macroscopic observation.

Similar to our result, Singh et al. (2017) isolated nine fungal isolates on TBA medium from different sources and were further used for quantitative production of lipase in submerged media. Kumar et al. (2012) isolated lipolytic fungal strain from common city garbage using tributyrin agar media as substrate. The isolate resulted in orange fluorescence under UV light on Rhodamine B olive agar media detecting lipase production.

Pandey et al. (2015) reported isolation of 80 fungal species from the samples followed by their screening on tributyrin agar media for identification of lipolytic fungi. Among these, only 10 fungal strains were demonstrated zone of hydrolysis on tributyrin agar media, indicated production of extracellular lipase. Among 6 fungi, 4 were identified as *Penicillium genus*, remaining 2 were identified as *Aspergillus* and *Alternaria* genus.

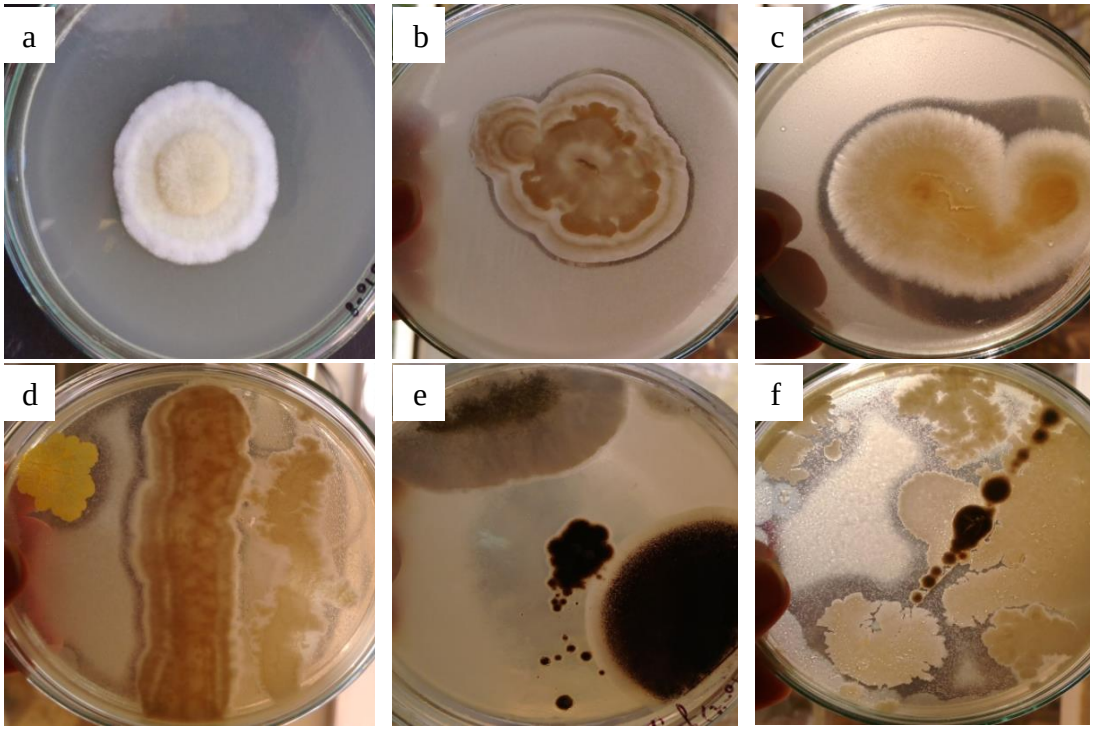

Figure 1. Formation of zone of hydrolysis on Tributyrin agar plates by fungal isolates named as (a): S3St2; (b): S6St1; (c): S3St3; (d): S2St1; (e): S3St1; (f): S4St2.

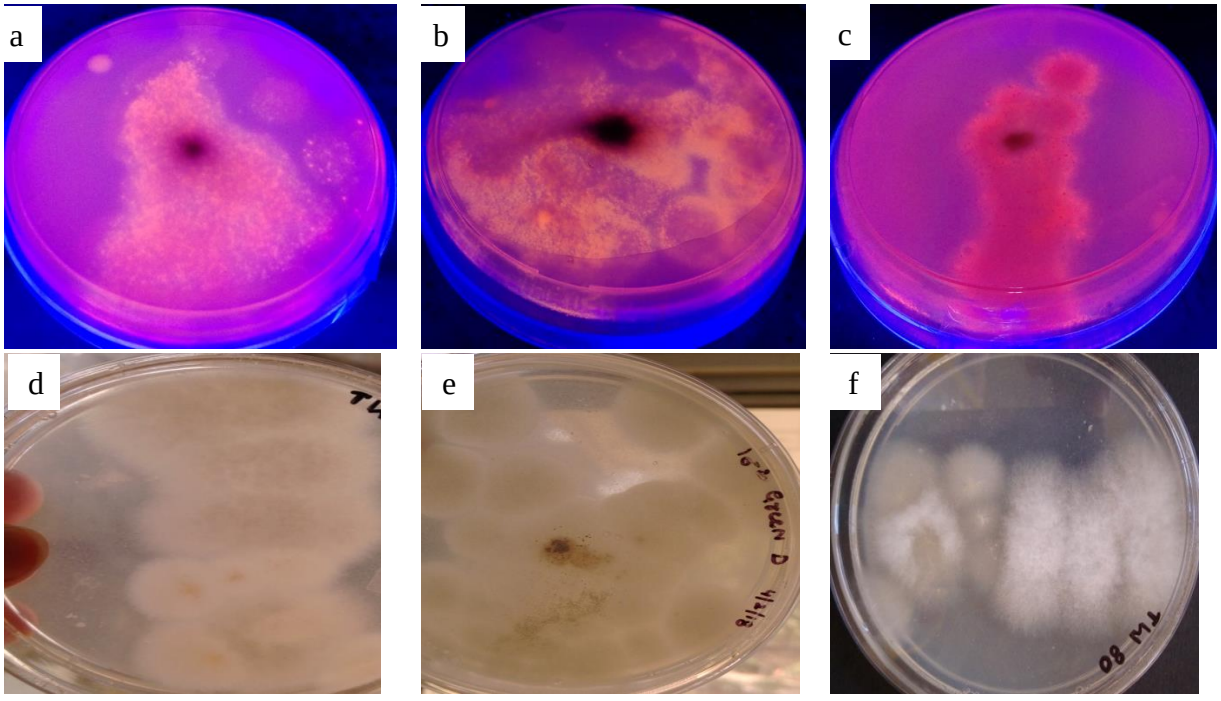

Figure 2. Formation of orange fluorescence on Rhodamine B agar plates by fungal isolates named as (a): S3St1; (b): S4St2; (c): S2St1; (exposed to UV at 350 nm) and Formation of white precipitation on Tween 80 agar plates by fungal isolates (d): S6St1; (e): S2St1; (f): S4St3.

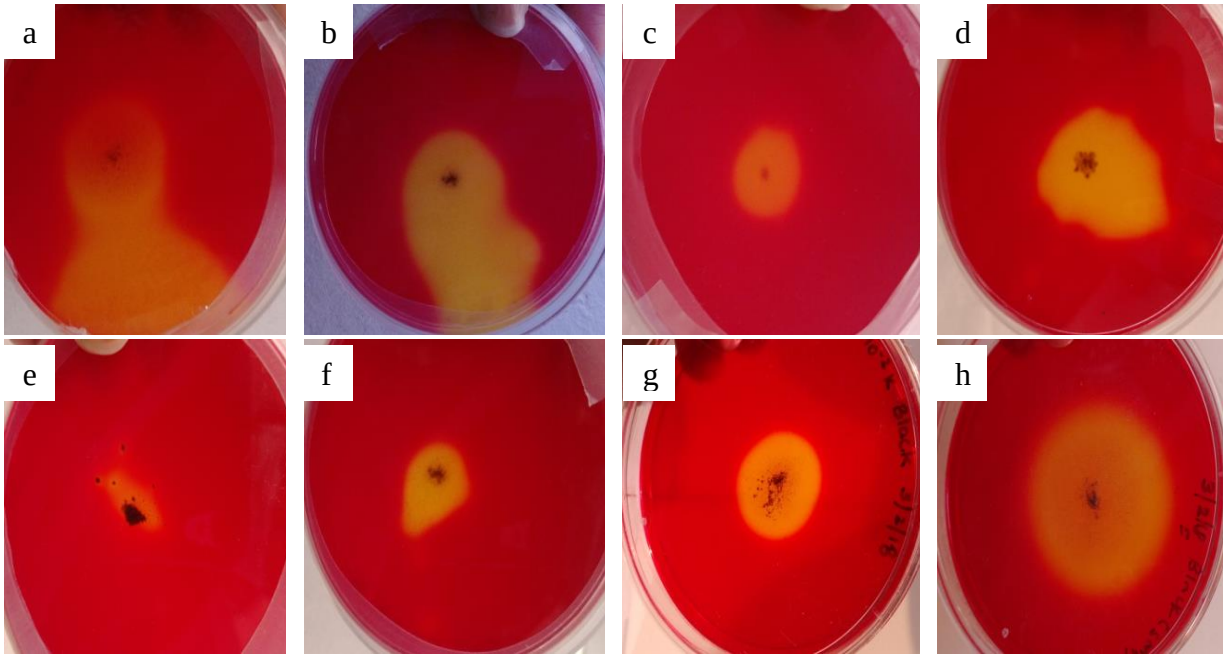

Figure 3. Formation of yellow color on phenol red agar plates by fungal isolates (a): S4St2; (b): S3St3; (c): S3St2; (d): S2St1; (e): S3St1; (f): S3St3; (g): S5St1; (h): S4St2.

CONCLUSION:

Samples were collected from 6 different sites. All samples were serially diluted and grown onto PDA plates. Fungal strains exhibited different characteristics and morphology. A total of 20 fungal isolates were subjected to screen onto four different media. 6 fungal isolates exhibited the zone of hydrolysis onto TBA plates, 3 fungal strain cultivated onto Rhodamine B plates exhibited orange fluorescent halos around fungal colonies visible under UV irradiation (350 nm), 8 fungal isolates demonstrated yellow color on Phenol red agar medium around their colonies and 3 fungal strains were identified by showing precipitation around colonies onto Tween 80 plates. Isolated strain which grown onto different media exhibited respective properties i.e are able to produce extracellular lipase. Submerged fermentation process will be used in further research for estimating lipolytic potential.

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