SCREENING AND ISOLATION OF LIPOLYTIC FUNGI FROM DIFFERENT SOURCES

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Abstract

Fungi were isolated from seven different sources. Soil sample was collected from the car workshop as fuel oil contaminated soil, Thuwana Township, Yangon Region, Myanmar. Other samples were collected from pork sausage, cheese, margarine, tea leaves in bean oil with salt, scraped coconut shell and scraped coconut peel. Fungal strains were directly isolated from 6 different sources. Diluted soil (concentration - 10^{-3}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}) was used to culture the fungi. Fungal strains were cultured on Potato Dextrose Agar (PDA) medium. Lipolytic fungi were screened using Tributyrin Agar (TBA) medium. Total twenty-five fungi were observed from seven different sources. Two fungal strains from pork sausage, five fungal strains from cheese, one fungal strain from margarine, two fungal strains from tea leaves in bean oil with salt, seven fungal strains from scraped coconut and eight fungal strains from fuel oil contaminated soil were observed. Among them, ten lipolytic fungi showed clear zone of hydrolysis around fungal colony on TBA medium that indicated lipase enzyme was produced. The isolated fungi were identified by their pure colony morphology and spore formation according to the references. In the present study, ten different types of lipolytic fungi were observed from six different sources.

Keywords: Lipolytic fungi, Different sources, Spore formation

Introduction

Lipase enzymes (Triacylglycerol acyl-hydrolase; EC 3.1.1.3) hydrolyze triacylglycerols which are the major constituents of fats and oils. Lipases catalyze the hydrolysis of long chain triacylglycerols to diacylglycerols, monoacylglycerols, fatty acids and glycerol. Lipase is a subclass of the esterases. Lipase enzyme has important roles in different biotechnological and industrial processes due to their diverse catalytic properties and substrate specificity. Their activities are used in the food-, pharmaceutical-, leather- and detergent industries as well as in the production of fine chemicals and biodiesel. Most of the current commercial enzymes are derived from microbial sources e.g. bacteria or filamentous fungi. The main advantage of enzyme production by microorganisms is that microorganisms can produce large amounts of enzyme economically (Alexandra, 2017).

Lipase producing microorganisms are in a wide range of environments such as industrial wastes, compost heaps, oilseeds, deteriorated food, vegetable oils processing factories and dairy products. Soil contaminated with oils also possesses a huge variety of enzymes producing microorganisms. Microorganisms are being used as lipase producers. Although plants, animals, fungi and bacteria widely produce lipase enzymes, fungal lipases are being used for various biotechnological purposes. Among those, filamentous fungi are considered as an ideal source of lipase production because they produce extracellular enzymes. *Aspergillus, Penicillium*, *Mucor*, *Rhizopus* and *Geotrichum* are the most luxuriant sources of lipase enzyme (Pandey *et al.*, 2016). Filamentous fungi are known as good lipase producers and numerous fungal enzymes are used in various food industrial processes. Since lipases produced by filamentous fungi are mainly extracellular, extraction and purification are relatively easy. This reason mentions to the fact that fungal lipases belong to the most important groups of commercial enzymes (Kotogan *et al.*,

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2014). In view of current and potential applications, lipases are considered to be a promising class of industrial enzymes (Kumar *et al.*, 2012).

This study was undertaken to isolate and identify the lipase producing fungi because of the numerous potential uses of lipase enzyme. The aim and objectives of this study were to screen lipolytic fungi from different sources and to identify lipolytic fungi into genus level according to their phenotypic characters and spore formation.

Materials and Methods

Sample preparation from different sources

1. Collection and isolation of lipolytic fungi from pork sausage (PSR)

Small pieces of pork sausage sample were kept in a plastic container. Pork sausage in the plastic container was incubated for two weeks until fungal growth was observed.

2. Collection and isolation of lipolytic fungi from cheese (CH)

Cheese was incubated in the plastic cup for eight days until fungal growth was observed.

3. Collection and isolation of lipolytic fungi from margarine (BM)

Small amount of margarine sample was kept in the plastic container. Margarine in the container was incubated for a month until fungal growth was observed.

4. Collection and isolation of lipolytic fungi from scraped coconut shell (CB)

Fungal growth was occurred on scraped coconut shell and taken from the coconut shop in the market.

5. Collection and isolation of lipolytic fungi from scraped coconut peel (SCP)

Scraped coconut peel in a petridish was incubated for thirteen days until fungal growth was observed.

6. Collection and isolation of lipolytic fungi from tea leaves in bean oil with salt (TOS)

Tea leaves in bean oil with salt were incubated in a container for five days until fungal growth was observed.

7. Collection and isolation of lipolytic fungi from fuel oil contaminated soil (OS)

Fuel oil contaminated soil sample was taken from the car workshop, Thuwana Township, Yangon Region, Myanmar. Soil sample was dried in the air. A ten-fold dilution series of soil was prepared according to Alexander and Strete (2001). The mixture of soil sample 1 g and 9 ml distilled water was carried out serial dilutions from 10^{-1} to 10^{-9}. After preparation of serial dilutions, 0.1 ml (100 uL) from selected dilutions was cultivated on Potato Dextrose Agar (PDA) medium plates and incubated at room temperature.

Fuel oil storage place at car workshop | Sample collected place | Collected soil sample | Soil sample was dried in the air

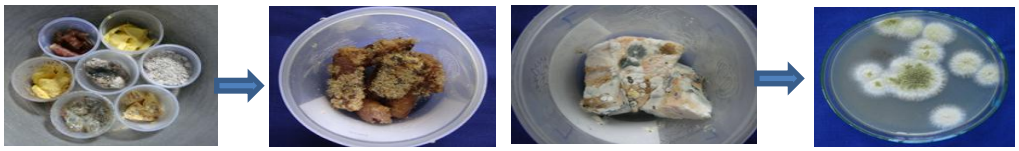
Figure 1 Soil sample collection from car workshop as fuel oil contaminated soil

Cultivation of Fungi

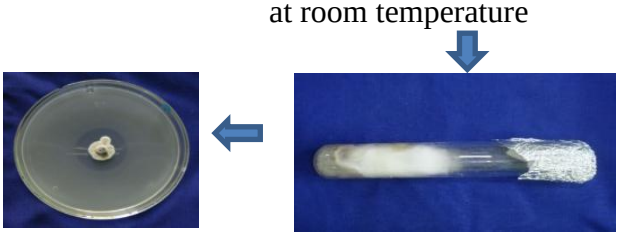
Fungal strains were directly collected from pork sausage, cheese, margarine, tea leaves in bean oil with salt, scraped coconut shell, scraped coconut peel and diluted soil contaminated with fuel oil. Fungi were cultivated and isolated on Potatoes Dextrose Agar (PDA) medium at room temperature for 5 - 7 days old. The pure fungal strains were maintained in test tubes with PDA medium. PDA medium was also used as stock culture medium or sub-culture medium for maintenance of fungus according to Atlas, 1993. All stock cultures were stored at 4 ºC. Potato Dextrose Agar (PDA) medium constituents: Mash Potato: 200 g, Peptone: 3 g, Dextrose: 20 g, Agar: 20 g, Distilled water: 1000 mL, pH: 6.5 ± 2 (Atlas, 1993). Chloramphenicol was added for antibacterial activity.

Screening of lipolytic fungi using Tributyrin Agar (TBA) medium

Screening of lipase producing fungi was done using tributyrin as a substrate on agar plates. Two different percentages (0.1 % and 1 %) of Tributyrin were used in this study. Lipolytic fungi were screened using Tributyrin Agar medium with 0.1 % tributyrin (Composition %/mL: Peptone: 0.5 g, Yeast extract: 0.3 g, Tributyrin (HiMedia): 0.1 mL, Agar: 2.0 g, pH: 6.0) according to Kotogan *et al.*, (2014) and Griebeler *et al.*, (2011). In addition, Tributyrin Agar (TBA) medium with 1 % tributyrin (composition %/mL: Peptone: 0.5 g, Yeast extract: 0.3 g, Agar: 2.0 g, Tributyrin (HiMedia): 1.0 mL, pH: 7.5 ± 0.2) was also used for screening of lipolytic fungi according to Wadia and Jain (2017). Clear hydrolytic halo regions occurred around colonies, it indicated that lipase enzyme was produced. All the isolated fungal cultures were inoculated on TBA plates and incubated at room temperatue for 2 - 17 days.



Different sources | Fungal growth on sources | Incubated for 5 days to 7 days with PDA medium at room temperature



Lipolytic fungi were screen on TBA medium | Pure strains were maintained in test tubes with PDA medium

Figure 2 Procedure of screening and isolation of lipolytic fungi from different sources

Identification of lipolytic fungi

Fungi were identified according to Barnett (1960) and Dube (1983).

Yeast Extract Agar medium

Yeast Extract Agar medium (Yeast extract: 3 g, Agar: 20 g, Distilled water: 1000 mL, pH: 7.0 ± 0.2. Chloramphenicol was added for antibacterial activity.) was used for direct microscopic examination of colony and spore formation according to Atlas, 1993. Lipolytic fungal strains were inoculated on Yeast Extract Agar medium plates and incubated at room temperature for 2 to 3 days before direct examination under microscope.

Results

Isolation of lipolytic fungi form different sources

Lipolytic fungi were isolated from seven different sources. In this study, ten different types of lipolytic fungi as shown in Table 1 and Figure 3 to 12 such as one *Aspergillus* sp. from pork sausage (Figure 3), one *Penicillium* sp. and one *Aspergillus* sp. from cheese(Figure 4 and 5), one *Aspergillus* sp. from tea leaves in bean oil with salt (Figure 6), one *Aspergillus* sp. from scraped coconut shell (Figure 7), one *Aspergillus* sp. from scraped coconut peel (Figure 8) and one *Monilia* sp. and three *Aspergillus* sp. from fuel oil contaminated soil (Figure 9, 10, 11 and 12) were observed. Each lipolytic fungus was identified based on their characters of pure colony morphology and spore formation according to Barnett (1960) and Dube (1983). The results were shown in Table 1 and Figure 3 to 12.

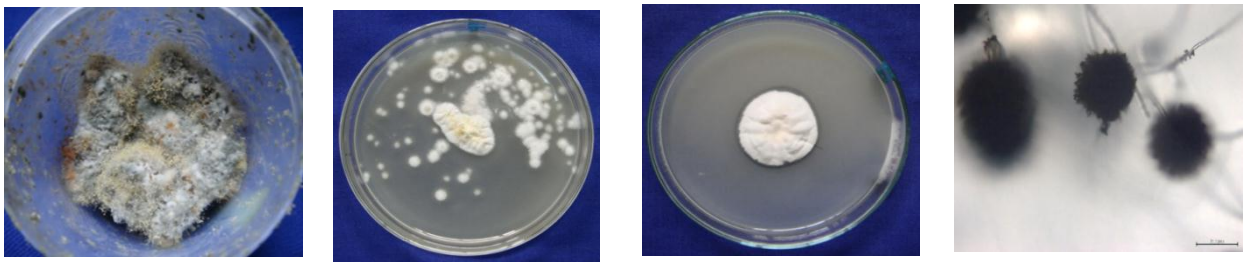
Table 1 Lipolytic fungi from different sources

No.	Fungal sources	Lipase producing fungi	Code of isolated strains	Clear zone/Halo region	
				0.1%TBA	1% TBA
1.	Pork sausage	*Aspergillus* sp. (1)	PSR-1	After 2-5 days	After 5- 17 Days
2.	Cheese	*Penicillium* sp.	CH-1	‖	‖
		Aspergillus sp. (2)	CH-3	‖	‖
3.	Tea leaves in bean oil with salt	*Aspergillus* sp. (3)	TOS-1	‖	‖
4.	Scraped coconut shell	*Aspergillus* sp. (4)	CB-5	‖	‖
5.	Scraped coconut peel	*Aspergillus* sp. (5)	SCP-4	‖	‖
6.	Fuel oil contaminated soil	*Monilia* sp.	OS-6	‖	‖
		Aspergillus sp. (6)	OS-3	‖	‖
		Aspergillus sp. (7)	OS-8	‖	‖
		Aspergillus sp. (8)	OS-13	‖	‖

Identification of isolated lipolytic fungi

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from Pork Sausage (PSR)

Aspergillus sp. (1) colony was yellow color inside and white color periphery. Mycelia were scattered in culture.

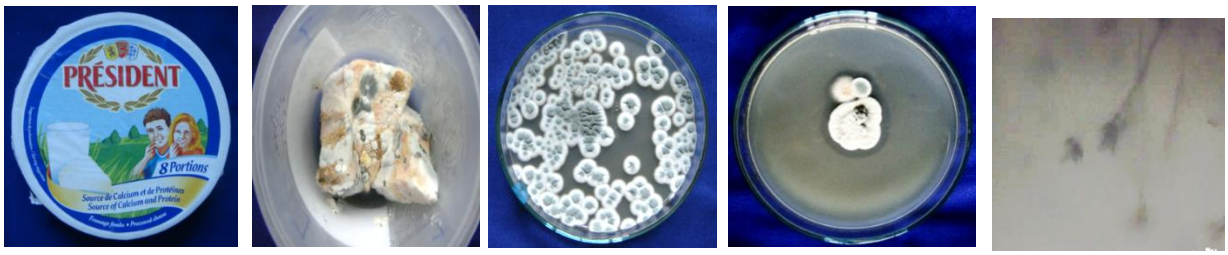


(A) **(B)** **(C)** **(D)**

Figure 3 Lipolytic fungus *Aspergillus* sp. (1) from pork sausage
(A) Fungal growth on pork sausage **(B)** Pure fungal colony (5 - 7 days old) (yellow color inside and white color periphery) (PSR - 1) **(C)** Clear zone (halo) around fungal colony (2 days old) on 1 % TBA medium **(D)** Micrograph of *Aspergillus* sp. (1) (X 200)

Characteristics of mycelium and spore formation of *Penicillium* sp. isolated from cheese (CH)

Penicillium sp. colony was green color inside and white color periphery. Mycelia were scattered in culture.

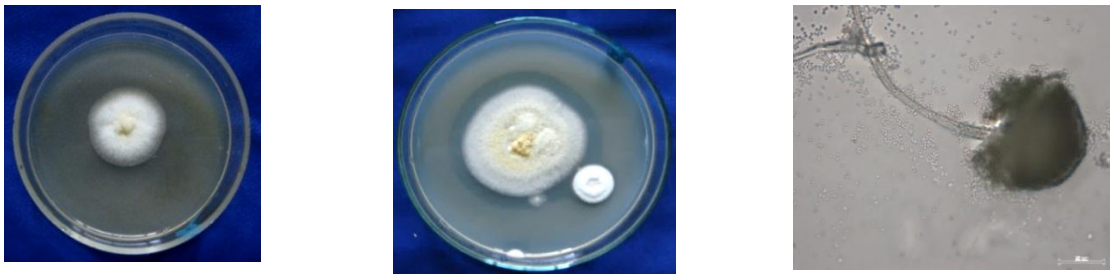


(A) **(B)** **(C)** **(D)** **(E)**

Figure 4 Lipolytic fungus *Penicillium* sp. (1) from cheese
(A) Collected cheese source **(B)** Fungal growth on cheese **(C)** Pure fungal colony (5-7 days old) (green color inside and white color periphery) (CH - 1) **(D)** Clear zone (halo) around fungal colony (5 days old) on 0.1 % TBA medium **(E)** Micrograph of *Penicillium* sp. (X 400)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from cheese (CH)

Aspergillus sp. (2) colony was yellow color inside and white color periphery. Mycelia were scattered in culture.

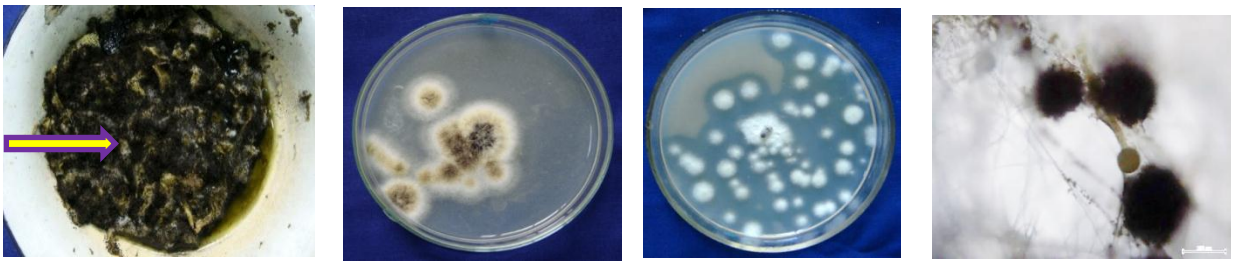


(A) **(B)** **(C)**

Figure 5 Lipolytic fungus *Aspergillus* sp. (2) from cheese **(A)** Pure fungal colony (5-7 days old) (yellow color inside and white color periphery) from cheese (CH.3) **(B)** Clear zone (halo) around fungal colony (5 days old) on 0.1% TBA medium **(C)** Micrograph of *Aspergillus* sp. (3) (X 400)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from tea leaves in bean oil with salt (TOS)

Aspergillus sp. (3) colony was black color inside yellow and white color periphery. Mycelia were scattered in culture.

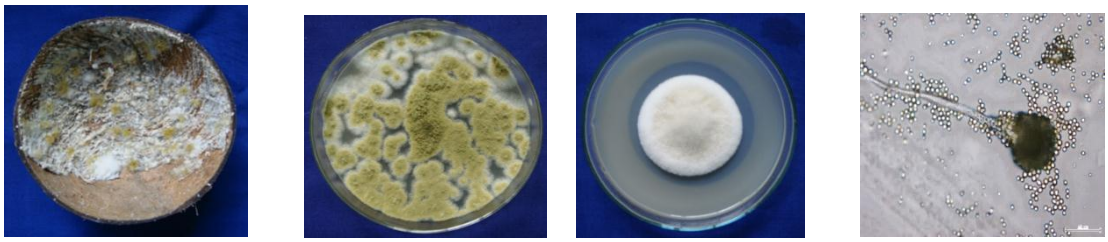


(A) **(B)** **(C)** **(D)**

Figure 6 Lipolytic fungus *Aspergillus* sp. (3) from tea leaves in bean oil with salt **(A)** Fungal growth on tea leaves in bean oil with salt **(B)** Pure fungal colony (5- 7 days old) (black color inside and yellow white color periphery) (TOS- 1) **(C)** Clear zone (halo) around fungal colony (7 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (3) (X 200)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from scraped coconut shell (CB.)

Aspergillus sp. (4) colony was green color inside and white color periphery. Mycelia were scattered in culture.

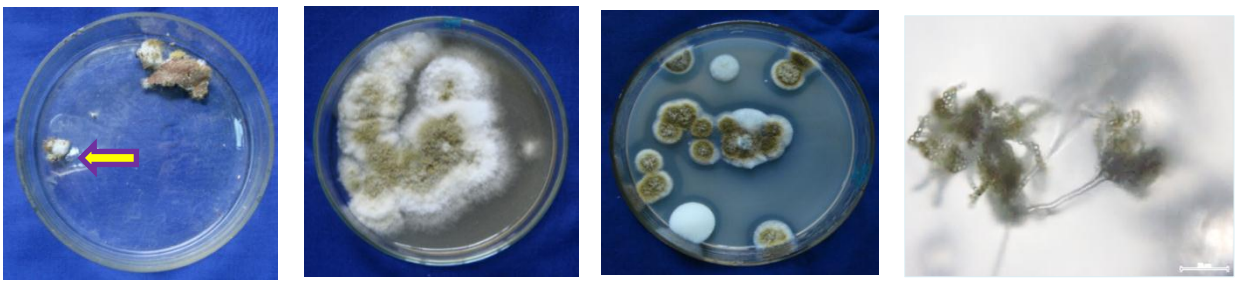


(A) **(B)** **(C)** **(D)**

Figure 7 Lipolytic fungus *Aspergillus* sp. (4) from scraped coconut shell
(A) Collected scraped coconut shell **(B)** Pure fungal colony (5 - 7 days old) (green color inside and white color periphery) (CB-5) **(C)** Clear zone (halo) around fungal colony (17 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (4) (X 400)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from scraped coconut peel (SCP)

Aspergillus sp. (5) colony was green color inside white color periphery. Mycelia were scattered in culture.



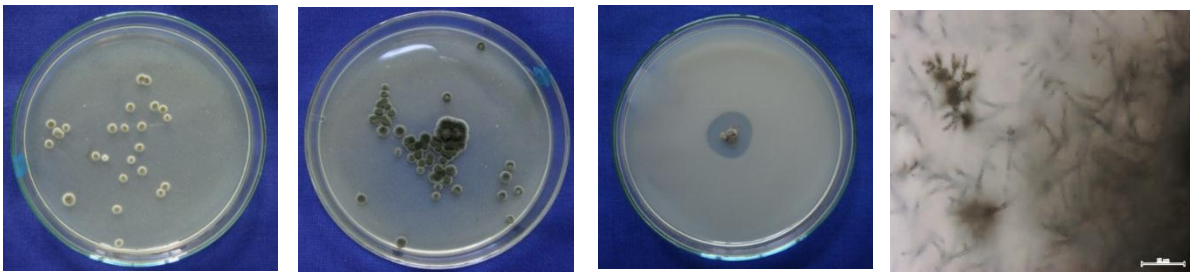
(A) **(B)** **(C)** **(D)**

Figure 8 Lipolytic fungus *Aspergillus* sp. (5) from scraped coconut peel

(A) Fungal growth on collected scraped coconut peel **(B)** Pure fungal colony (5 - 7 days old) (green color inside and white color periphery) (SCP.4) **(C)** Clear zone (halo) around fungal colony (7 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (5) (X 400)

Characteristics of mycelium and spore formation of *Monilia* sp. isolated from fuel oil contaminated soil (OS)

Monilia sp. colony was black green color inside and white color periphery.



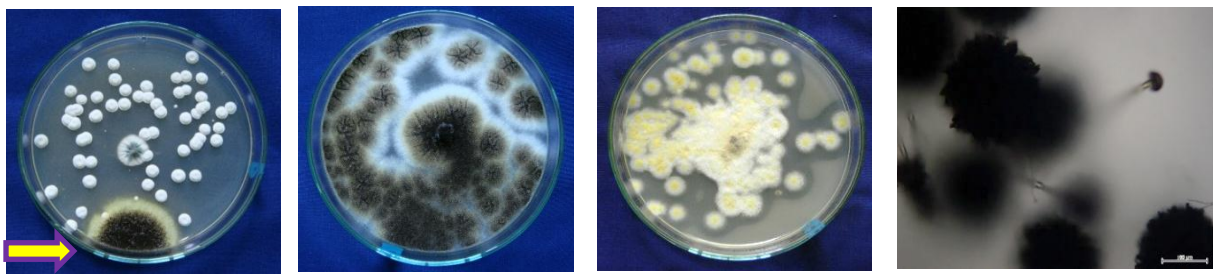
(A) **(B)** **(C)** **(D)**

Figure 9 Lipolytic fungus *Monilia* sp. from fuel oil contaminated soil

(A) Fungus isolated from soil sample (10^{-6}) **(B)** Pure fungal colony (5-7 days old) (black green color inside and white color periphery) from soil sample (10^{-6}) (OS.6) **(C)** Clear zone (halo) around fungal colony (17days old) on 1% TBA medium **(D)** Micrograph of *Monilia* sp. (X 400)

Characteristics of mycelium and spore formation of *Aspergillus* sp. isolated from fuel oil contaminated soil (OS)

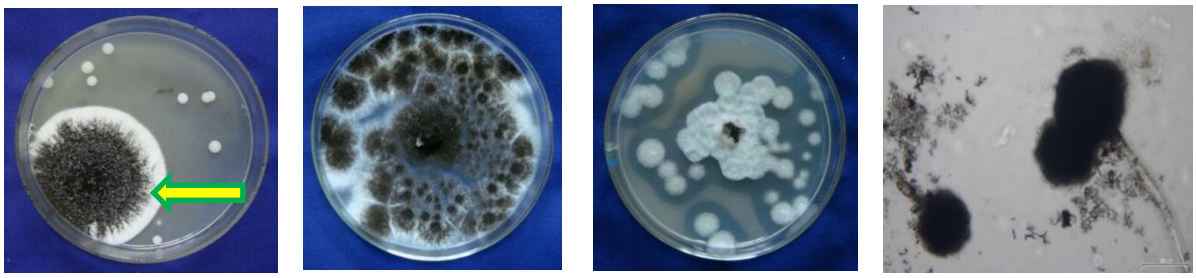
Aspergillus sp. (6) colony was black color inside and yellow white color periphery. Mycelia were scattered in culture. *Aspergillus* sp. (7) colony was black color inside and white color periphery. Mycelia were scattered in culture. *Aspergillus* sp. (8) colony was yellow color inside and white colony periphery. Mycelia were scattered in culture.



(A) **(B)** **(C)** **(D)**

Figure 10 Lipolytic fungus *Aspergillus* sp. (6) from fuel oil contaminated soil

(A) Fungus isolated from soil sample (10^{-3}) **(B)** Pure fungal colony (5-7 days old) (black color inside and yellow white color periphery) from soil sample (10^{-3}) (OS.3) **(C)** Clear zone (halo) around fungal colony (6 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (6) (X 200)



(A) **(B)** **(C)** **(D)**

Figure 11 Lipolytic fungus *Aspergillus* sp. (7) from fuel oil contaminated soil
(A) Fungus isolated from soil sample (10^{-8}) **(B)** Pure fungal colony (5-7 days old) (black color inside and white color periphery) from soil sample (10^{-8}) (OS.8) **(C)** Clear zone (halo) around fungal colony (6 days old) on 1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (7) (X 200)

(A) **(B)** **(C)** **(D)**

Figure 12 Lipolytic fungus *Aspergillus* sp. (8) from fuel oil contaminated soil

(A) Fungus isolated from soil sample (II. 10^{-7}) **(B)** Pure fungal colony (5-7 days old) (yellow color inside and white color periphery) from soil sample (II. $10^{-7)}$ (OS.13) **(C)** Clear zone (halo) around fungal colony (5 days old) on 0.1% TBA medium **(D)** Micrograph of *Aspergillus* sp. (8) (X 200)

Table 2 Characters of lipolytic fungi isolated from different sources

Sr. No.	Fungal sources	Code of isolated strains	Macroscopic characters of lipolytic fungi	Microscopic characters of lipolytic fungi	Species
1.	Pork sausage	PSR-1	Mycelium was yellow color inside and whit color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (1)
2	Cheese	CH-1	Mycelium was green color inside and white color periphery. Mycelia were scattered in culture.	Conidiophores arising from the mycelium singly, branched near the apex to form a brush-like, conidia-bearing apparatus, conidia brightly colored in mass, 1-celled, mostly globose or ovoid, produced basipetally.	*Penicillium* sp.

Sr. No.	Fungal sources	Code of isolated strains	Macroscopic characters of lipolytic fungi	Microscopic characters of lipolytic fungi	Species
3.		CH-3	Mycelium was yellow color inside and white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (2)
4.	Tea leaves in bean oil with salt	TOS-1	Mycelium was black color inside yellow and white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (3)
5.	Scraped coconut shell	CB-5	Mycelium was green color inside and white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (4)
6.	Scraped coconut peel	SCP-4	Mycelium was green color inside white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose, radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (5)
7.	Fuel oil contaminated soil	OS-6	Mycelium was black green color inside and white color periphery.	Mycelium white or gray, abundant in culture; conidia gray or tan in mass, 1-celled, short cylindric to rounded, catenulate, formed acropetally, conidiophore branched.	*Monilia* sp.
8.		OS-3	Mycelium was black color inside and yellow white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (6)
9.		OS-8	Mycelium was black color inside and white color periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (7)
10.		OS-13	Mycelium was yellow color inside and white colony periphery. Mycelia were scattered in culture.	Conidiophores upright, simple, terminating in a globose radiating from the entire surface; conidia 1-celled, globose, catenulate, produced basipetally.	*Aspergillus* sp. (8)

Discussion and Conclusion

In this research work, screening, isolation and identification of lipase producing fungi were studied from different sources. Total twenty-five fungi were observed from seven different sources. Two fungal strains from pork sausage, five fungal strains from cheese, one fungal strain from margarine, two fungal strains from tea leaves in bean oil with salt, seven fungal strains from scraped coconut and eight fungal strains from fuel oil contaminated soil were observed. Among them, ten different types of lipolytic fungi were observed from six different sources.

In this study, lipolytic fungi such as different species of *Aspergillus* from pork sausage, scraped coconut shell, scraped coconut peel, tea leaves in bean oil were observed. *Penicillium* sp. and *Aspergillus* sp. were observed from cheese. Zohri *et al.* (2014) also reported that *Aspergillus niger*, *A. terreus*, *A. flavus*, *Penicillium chrysogenum* and *P. citrinum* were the most common fungal species on both beef burger and sausage samples. All beef burger and sausage samples were contaminated with different fungal species. Most isolated fungi were able to produce lipase enzyme.

In the present research, three *Aspergillus* species and *Monilia* sp. were observed from fuel oil contaminated soil. Colla *et al.* (2015) described that lipases from different sources may present different properties. Two *Aspergillus* species were isolated from the diesel-contaminated soil in the city of Passo Fundo, RS, Brazil and selected as good lipase producers. In addition, Mukhtar *et al.* (2015) also stated that seven different lipolytic fungal strains were found from soil samples for lipase production. Among those fungal strains, *Aspergillus niger* revealed the best results.

Fattah and Hammad (2002) reported that filamentous fungi from soil were screened and isolated for extracellular lipase production. Among ten isolated fungi, *Aspergillus niger* and *Aspergillus terreus* revealed as the highest lipase producers. Sumathy *et al.* (2012) also reported that *Aspergillus*, *Penicillium*, *Mucor*, *Geotrichum*, *Fusarium* and *Rhizopus* genera were widely used as sources of lipases.

In this study, three different genera of lipolytic fungi were observed from six different sources. They were *Aspergillus, Penicillium and Monilia* genera. Lipolytic fungi such as eight *Aspergillus* species, one *Penicillium* sp. and one *Monilia* species from pork sausage, cheese, scraped coconut shell, scraped coconut peel, tea leaves in bean oil and fuel oil contaminated soil were observed. Total ten lipolytic fungi were screened and isolated from six different sources. Among them, about 4 to 8 lipolytic fungi with the most outstanding clear zone will be selected for the future study.

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