Comparison of lipase enzyme activities extracted from lipolytic fungi

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Abstract

Lipolytic fungi were isolated from scraped coconut shell and fuel oil-contaminated soil. The isolated fungal strains were screened on tributyrin agar (TBA) for exogenous lipolytic activity. The screened lipolytic fungi were identified by their pure colony morphology and spore formation according to the references. Total 11 lipolytic fungi were isolated from two different sources. In this study, two lipolytic fungi were selected based on large clear zone of hydrolysis (halo) on TBA medium for further study; they were *Aspergillus* sp. (11) isolated from scraped coconut shell (CS) and *Aspergillus* sp. (18) from fuel oil contaminated soil (OS), respectively. The isolates were tested for lipolytic activity in the submerged fermentation (SmF) medium by spectrophotometry using p-nitrophenyl palmitate as substrate. Crude lipase was produced using optimal parameters and partially purified using ammonium sulfate followed by desalting with a dialysis membrane. The hydrolysis activity of lipase was investigated by spectrophotometric determination and TLC methods using olive oil as a substrate. According to the results of TLC separation, the partially purified lipases produced by *Aspergillus* spp. (11 and 18) were non-specific lipases. Moreover, the degradation of lipids by crude lipases from *Aspergillus* spp. (11 and 18) was tested using different vegetable oils compared with distilled water. Lipase activity (removing oil stains) was also investigated by using a detergent for industrial applications. As the research has demonstrated, partially purified lipases produced by *Aspergillus* spp. (11 and 18) had exploitable properties for industrially relevant applications, especially removing oil stains and lipid degradable applications.

Keywords: Lipase, lipolytic fungi, *Aspergillus*, removing oil stains

Introduction

Lipase is considered as an important biocatalyst since it has numerous applications in different industrial processes (Lanka and Trinkle, 2017). Lipase enzymes (Triacylglycerol acyl-hydrolase; EC 3.1.1.3) catalyze the hydrolysis of triacylglycerols, which are the major constituents of fats and oils. Lipases are ubiquitous enzymes and are produced from various sources like plants, animals, and microorganisms (Niaz *et al.*, 2014). Microorganisms are sources for most of the current commercial enzymes due to high productivity, genetic modifiability, and low costs (Wadia and Jain, 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 enzyme-producing microorganisms (Pandey *et al.*, 2016). Although plants, animals, fungi, and bacteria widely produce lipases, fungal lipases are being used for various biotechnological purposes. 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. Fungal lipases are important for different industrial purposes because of their distinctive properties and ease of mass production. Therefore, the importance of fungal lipase research has enormously increased (Pandey *et al.*, 2016).

Lipases hydrolyze the long-chain triacylglycerols to di-acylglycerols, mono-acylglycerols, fatty acids, and glycerol (Alexandra, 2017). According to their

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specificity, lipases can be divided into two groups such as 1,3-positional specific lipase and non-positional-specific lipase (Helal *et al.*, 2021). Lipases have promising applications in detergent formulation, bioremediation, food, flavor industry, organic chemical processing, agrochemical industry, pharmacy, paper manufacturing, cosmetics, and perfumery (Houde *et al.*, 2004 and Jagdish *et al.*, 2013). The major industrial applications of lipase enzyme are in detergent production due to its remarkable ability in removing oil stains from fabrics (Alabdalall *et al.*, 2021). Especially in the detergent industry, lipases are formulated with detergents to overcome using high temperature in the washing which consumes higher energy. Moreover, lipases are able to maintain the quality of texture and fabrics (Helal *et al.*, 2021).

This study investigated the screening of lipolytic fungi, lipase production, partial purification, and their potential activities as degradation of lipids and removing of oil stains for detergent industry.

Materials and Methods

Sample preparation from different sources

1. Collection and isolation of lipolytic fungi from scraped coconut shell (CS)

Fungal-contaminated coconut shell, scraped coconut shell, was taken from a coconut shop in a local market.

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

Fuel oil-contaminated soil sample was taken from the ground soil of 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). After preparation of serial dilutions, 0.1 mL 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 the ground soil of car workshop as fuel oil-contaminated soil

Cultivation of fungi

Fungal strains were directly collected from scraped coconut shell and diluted soil contaminated with fuel oil. Fungi were cultivated on Potato Dextrose Agar (PDA) medium at room temperature for 5 - 7 days according to Atlas, 1993. The pure fungal strains were maintained in test tubes with Czapek-Dox Agar medium. Czapek-Dox Agar medium was used as stock culture medium or sub-culture medium for maintaining the fungus according to the method of Raper and Thom, 1945. Chloramphenicol was added for antibacterial activity.

Screening of lipolytic fungi using Tributyrin Agar (TBA) medium

Tributyrin Agar (TBA) medium was used for the screening of lipolytic fungi. 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 (HiMedia) was also used for screening of lipolytic fungi according to Wadia and Jain (2017). The isolated fungal cultures were inoculated on TBA medium plates and incubated at room temperature for 5 - 17 days. The formation of clear hydrolytic halo regions around colonies indicated the production of lipase.

Identification of isolated lipolytic fungi

Fungi were identified according to Barnett and Hunter (1998) and Dube (1983).

Extraction of enzyme from fermentation broth

All media were examined by taking fermented broth (10 mL) in 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, and 7 days of fermentation to observe the best fermentation period for lipase production. The filtrates were centrifuged at 10,000 rpm, 4˚C for 10 minutes to obtain supernatant. The clear supernatant was considered as crude enzyme. The resulting supernatant was evaluated for lipase activity by using p-nitrophenyl palmitate (pNPP, Sigma) as a substrate as described by Winkler and Stuckmann, 1979 (Massadeh and Sabra, 2011, Pandey *et al.*, 2016, and Rodrigues *et al.*,2016).

Lipase activity assay

The extracellular lipase activity was determined by using p-nitrophenyl palmitate (p-NPP) (Sigma, USA) as substrate according to Winkler & Stuckmann (1979). The pNPP substrate solution was prepared by freshly mixing solution A (3 mg of pNPP in 1 mL of isopropanol) with solution B (10 mg of gum Arabic and 40 µL of Triton X-100 in 9 mL of Tris-HCl buffer, pH 8.0) while stirring until all was dissolved. One mL of freshly prepared p-NPP solution was incubated in a water bath at 37ºC for 10 minutes. After 10 min, 0.5 mL of crude enzyme sample and 0.5 mL of Tris-HCl buffer were added and the reaction mixture was further kept in the water bath for 30 min at 35-37ºC. After that, the enzymatic reaction was stopped by adding 0.1 mL of 100 mM $CaCl_2.2H_2O$. The formation of a yellow color due to the release of p-nitrophenol indicated lipase activity. The absorbance of the yellow color was measured by spectrophotometry at 410 nm against a control without enzyme (Massadeh and Sabra, 2011, and Pandey *et al.* 2016). The concentration of liberated yellow color compound (p-nitrophenol) in the reaction mixture was determined by using the standard curve of p-nitrophenol (2 to 12 µg mL^{-1} in 0.05 M Tris HCl buffer, pH-8.0). One unit (U) of lipase activity was defined as micromole (µM) of p-nitrophenol liberated from the hydrolysis of p-nitrophenyl ester by one mL of soluble enzyme per minute at 37°C under standard assay conditions (Kanwar *et al.*, 2005).

$$\text{Lipase activity (Units ml}^{-1}\text{ min}^{-1}\text{)} = \frac{\mu\text{moles of p-nitrophenol liberated}}{\text{Volume of enzyme taken (0.5 mL)} \times \text{Incubation time (30 min)}}$$

Preparation of p-nitrophenol standard curve: The standard curve of p-nitrophenol was prepared using the concentration range of 2 - 12 µg ml^{-1} of p-nitrophenol in 0.05 M Tris HCl buffer, pH-8.0 according to Kanwar *et al.* (2005).

Partial purification of lipase enzyme by ammonium sulfate precipitation

Lipases produced by *Aspergillus* spp. (11) and (18) under Smf were partially purified using ammonium sulfate followed by dialysis. Ammonium sulfate precipitation was done according to Basheer (2007), Singh *et al.* (2014), Nayak (2013), Jagdish *et al.* (2013) and Anilkumar (2020).

Dialysis (Desalting of enzymes)

The procedure of dialysis was performed according to Anilkumar (2020) and Kashmiri *et al.* (2006). The precipitates obtained after ammonium sulfate precipitation

were further dialyzed against Tris-HCl buffer in order to remove ammonium sulfate from the precipitates. The precipitate was resuspended in minimal amount (recorded the volume of added buffer solution) of 0.05 M Tris-HCl buffer (pH 8.0). The redissolved protein solution in a dialysis bag (molecular weight cut-off value 12-14 kDa) was dialyzed against Tris-HCl buffer (0.05 M, pH 8.0) for 4 h at room temperature under continuous stirring using a magnetic stirrer. The buffer was replaced with fresh buffer after every 2 h. After 4 h, the dialysis was continued overnight at 4ºC. The dialysate was collected in a sterile container and the volume of dialysate was measured.

Protein content and specific activity

Protein assay

Protein content of the crude sample extract was estimated by Lowry method using Bovine Serum Albumin (BSA) as the standard (Lowry *et al.*, 1951).

Preparation of BSA standard curve

A standard curve of BSA was drawn in the following concentration range: 40, 80, 120, 160, and 200 μg mL^{-1} (Basheer, 2007). Different volumes (0.2, 0.4, 0.6, 0.8 and 1 mL) of working standard solution were pipetted out into a series of test tubes while keeping one test tube blank.

Calculation of specific activity and enzyme purification (fold)

Specific activity and purification fold were calculated according to Helal *et al.* (2021).

Determination of hydrolysis activity of lipase

The free fatty acids (FFA) liberated by the hydrolytic action of lipase were determined by two methods such as spectrophotometric determination according to Marseno *et al.* (1998) and H-Kittikum *et al.* (2021), and Thin Layer Chromatography (TLC) method according to Autryve *et al.* (1991), Marseno *et al.* (1998), Lu *et al.* (2009), and Holzl and Dormann (2021).

Standard curve of free fatty acid

The standard curve of free fatty acid was prepared according to the method of Marseno *et al.*, 1998. The standard curve of oleic acid was prepared by dissolving oleic acid in isooctane to give a series of concentration of 2-10 μmole/2mL in test tubes. Each concentration of oleic acid was dissolved in isooctane to total 2 mL while keeping one test tube blank. Then 0.4 mL of 5% cupric acetate-pyridine (pH 6.0) was added. Contents in each tube were mixed vigorously for 90 sec by hand. The mixture was centrifuged* for 2 min at 2,000 rpm. Fatty acid content in the supernatant phase (isooctane fraction) was measured by spectrophotometer at 715 nm. (*The centrifugation step can be omitted and replaced by mixing for 5 sec using a vortex mixer and allowed for 10 min.)

Preparation of reaction mixture for spectrophotometric determination

60% (v/v) olive oil in isooctane (reaction mixture): 60 mL of olive oil was mixed with 40 mL of isooctane.

5% (w/v) cupric-acetate pyridine (pH 6.0): 5 g of copper acerate was solubilized in 80 mL of distilled water and the pH was adjusted to 6.0 using pyridine. Then, final volume was adjusted to 100 mL using distilled water.

Procedure: One mL of lipase solution was added to 2 mL of reaction mixture in a conical flask (50 mL). The mixture was agitated on a rotary shaker, 300 rpm, 30 min at room temperature. After 30 min, the reaction was stopped by placing the mixture in an ice bath for a few minutes. Then, 200 μL of the aliquots was added to the mixture containing 1800 μL of isooctane and 400 μL of 5% cupric-acetate pyridine (pH-6.0). The content of free fatty acids in the mixture was measured by a spectrophotometer at

715 nm and determined by using a standard curve of oleic acid. One unit of lipase activity was defined as the amount of enzyme that produced 1 μmole of fatty acids per minute.

Preparation of reaction mixture for TLC method

60% (v/v) olive oil in isooctane (reaction mixture): 60 mL of olive oil was mixed with 40 mL of isooctane.

Solvent system: The solvent mixture was n-hexane/diethyl ether/acetic acid in a ratio of 70:30:1 (v/v/v). A glass tank was filled with 100 mL of the solvent. The tank was closed and waited until it was equilibrated with solvent vapor.

Iodine vapor: 5 g of crystalline iodine was added to a plastic container with a lid. Iodine was allowed to equilibrate for a few hours so that the iodine vapor could fill the entire tank. The spots were revealed by iodine vapor.

TLC plate: 20 x 20 cm Silica gel 60 F254 -Merck, Germany

Procedure: One mL of lipase solution and 2 mL of lipase solution were separately added to 2 mL of reaction mixture in each conical flask (50 mL). The mixture was shaken on a rotary shaker, 300 rpm, 30 min at room temperature. After 30 min, the reaction was stopped by placing the mixture in an ice bath for a few minutes. Sample was loaded onto a silica gel (60 F_{254} -Merck) plate, about 1.5 cm from the bottom of the plate, using a capillary tube. Reference lipids such as oleic acid (commercial), oleic acid from HiMedia, and olive oil (extra virgin) were also loaded in separate lanes. After drying the lipid spots, the silica gel plate (20 cm x 20 cm) was placed in the glass tank and the tank was closed. The plate was developed until the solvent front reached a distance of about 1.5 cm from the top of the plate. The TLC plate was removed from the tank and dried it in an oven for few minutes. The dry TLC plate was placed in an iodine vapor tank for 5 - 15 min to develop brown-colored spots. The plate was removed from the tank. A yellowish to brownish color of spots was marked with a pencil and measured.

Application Studies

Degradation of lipid

For qualitative determination of lipase activity, crude lipases were tested for their efficiency in degrading vegetable oil according to Alabdalall *et al.* (2021), Affes *et al.* (2017), and Facchini *et al.* (2015) with some modifications. The hydrolysis of lipid was performed with various vegetable oils (olive oil, peanut oil, and coconut oil). Two hundred microliter of each oil was added to a beaker containing 2 mL crude enzyme. The reaction was incubated on a rotary shaker at 300 rpm, room temperature over 4 to 8 days. The hydrolysis activity was examined every day. Distilled water with each oil sample was used as a control.

Removing oil stains

Application of partially purified lipase as a detergent additive was studied according to Helal *et al.* (2021), Alabdalall *et al.* (2021), Sharma *et al.* (2017), and Soler (2019) with some modifications. Sue-Sat powder detergent (Thailand) was used in this experiment. The partially purified lipases were determined for their efficiency in removing oil stains from fabrics. A polycotton fabric was cut into pieces (6×6 cm). Each piece was stained separately with 100 μL of olive oil, fried oil (used to fry fish), and vehicle oil (used engine oil). Then, fabric pieces were allowed to dry. Each oil-stained fabric was placed in 100 mL flasks with four different treatments.

Four treatments were: (a) distilled water (50 mL), (b) distilled water and detergent (49 mL + 1 g), (c) water and lipase (48 mL + 2 mL), (d) water with lipase and detergent (47 mL + 2 mL + 1 g). The treatment was incubated and gently agitated for 30 min. Then, fabric pieces were removed and dried. After drying, the fabric piece was examined for the presence of oil stain residues.

Results

Isolation and identification of lipolytic fungi from different sources

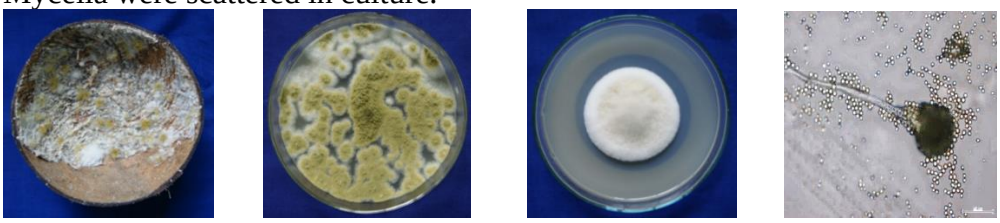
Lipolytic fungi were isolated from different sources. In this study, two different types of lipolytic fungi as shown in Table 1 such as *Aspergillus* sp. (11) (was selected from 3 strains) from scraped coconut shell (Figure 2) and *Aspergillus* sp. (18) (was selected from 8 strains) from fuel oil contaminated soil (Figure 3) were observed. Each lipolytic fungus was identified based on its characteristics of pure colony morphology and spore formation according to Barnett and Hunter (1998) and Dube (1983).

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.	Scraped coconut shell	*Aspergillus* sp. (11)	CS-5	After 2-5 days	After 5- 17 days
2.	Fuel oil-contaminated soil	*Aspergillus* sp. (18)	OS-8	‖	‖

Characteristics of mycelium and spore formation of *Aspergillus* sp. (11) isolated from scraped coconut shell (CS)

Aspergillus sp. (11) colony was green color inside and white color periphery. Mycelia were scattered in culture.

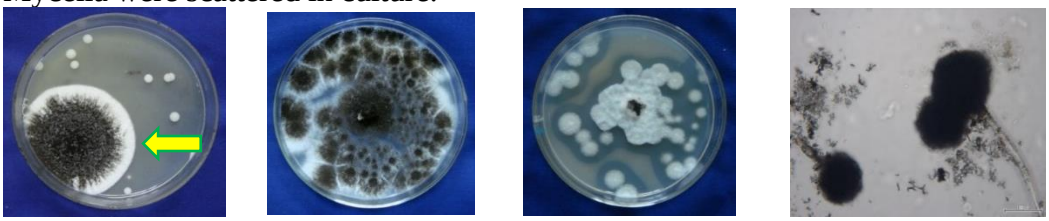


(A) (B) (C) (D)

Figure 2. Lipolytic fungus *Aspergillus* sp. (11) from scraped coconut shell
(A) Collected scraped coconut shell (B) Pure fungal colony (5 - 7 days old) (green color inside and white color periphery) (CS-5) (C) Clear zone (halo) around fungal colony (17 days old) on 1% TBA medium (D) Micrograph of *Aspergillus* sp. (11) (X 200)

Characteristics of mycelium and spore formation of *Aspergillus* sp. (18) isolated from fuel oil-contaminated soil (OS)

Aspergillus sp. (18) colony was black color inside and white color periphery. Mycelia were scattered in culture.



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

Figure 3. Lipolytic fungus *Aspergillus* sp. (18) 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})

(C) Clear zone (halo) around fungal colony (6 days old) on 1% TBA medium
(D) Micrograph of *Aspergillus* sp. (18) (X 400)

Partial purification of lipase from *Aspergillus* sp. (11)

Crude lipase produced by *Aspergillus* sp. (11) was partially purified by ammonium sulfate precipitation followed by dialysis and the results were shown in Table 2. The specific activity (1.93 U/mg) with 40.05-fold was recorded at 30-70% ammonium sulfate fractionation. After the dialysis process, the specific activity showed 2.30 U/mg, with 47.82-fold increase, respectively.

Table 2. Partial purification of lipase from *Aspergillus* sp. (11) using various concentrations of Ammonium sulfate and dialysis

Purification step	Lipase activity (U/mL)	Protein (mg/mL)	Specific activity (U/mg)	Purification (fold)
Crude lipase	0.04	0.88	0.05	1
Supernatant after ammonium sulfate fractionation 30%	0.19	0.73	0.26	5.45
Ammonium sulfate fractionation 70%	0.90	0.47	1.93	40.05
Dialysate	19.52	8.48	2.30	47.82

Partial purification of lipase from *Aspergillus* sp. (18)

Crude lipase (0.04 U/mg) produced by *Aspergillus* sp. (18) was partially purified by ammonium sulfate precipitation followed by dialysis and the results were shown in Table 3. The result of partially purified lipase showed the specific activity of 0.15 U/mg and 3.88-fold of purification.

Table 3. Partial purification of lipase from *Aspergillus* sp. (18) using various concentrations of Ammonium sulfate and dialysis

Purification step	Lipase activity (U/mL)	Protein (mg/mL)	Specific activity (U/mg)	Purification (fold)
Crude lipase	0.20	5.40	0.04	1
Supernatant after ammonium sulfate fractionation 30%	0.39	4.12	0.09	2.48
Ammonium sulfate fractionation 70%	2.29	21.95	0.10	2.76
70% Dialysate	1.34	9.16	0.15	3.88

Determination of Hydrolysis Activity of Lipase

Free fatty acid (FFA) released from the hydrolysis activity of lipases was confirmed by two methods such as spectrophotometric (715 nm) method and Thin-layer chromatography (TLC) method.

Determination of hydrolysis activity of lipase by spectrophotometric method

Olive oil as a substrate was hydrolyzed by using partially purified lipase (enzyme unit of 19.52 U/mL) by *Aspergillus* sp. (11), and 1.34 U/mL by *Aspergillus* sp. (18) for 30 min and the form of free fatty acid (oleic acid) liberated from olive oil was shown in Table 4. *Aspergillus* sp. (11) exhibited the hydrolysis activity of lipase (2.53 U/mL) while *Aspergillus* sp. (18) showed the hydrolysis activity (2.73 U/mL) compared to other strains.

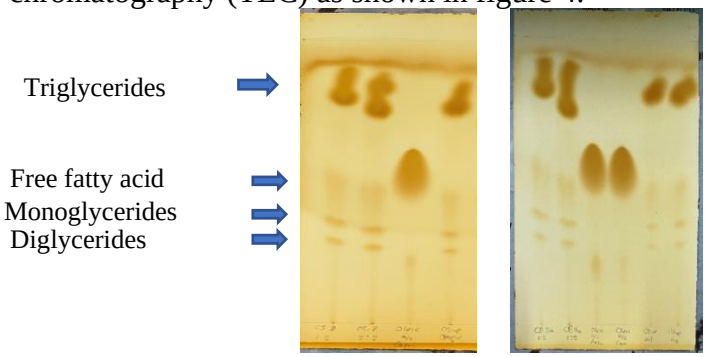
Table 4. Determination of oleic acid production by lipase at 715 nm

Sr. no	**Strain**	Ammonium sulphate fractionation	Enzyme	**Lipase activity (U/ml)**
1	***Aspergillus* sp. (11)**	70% Dialysate	Partially purified	2.53

2	***Aspergillus* sp. (18)**	70% Dialysate	Partially purified	2.73

Determination of hydrolysis activity of lipase by Thin Layer Chromatography method

Hydrolysis activity of partially purified lipase (enzyme unit of 19.52 U/mL) produced by *Aspergillus* sp. (11) and 1.34 U/mL by *Aspergillus* sp. (18) was determined via hydrolysis products of olive oil (as a substrate) through Thin-layer chromatography (TLC) as shown in figure 4.



(B)

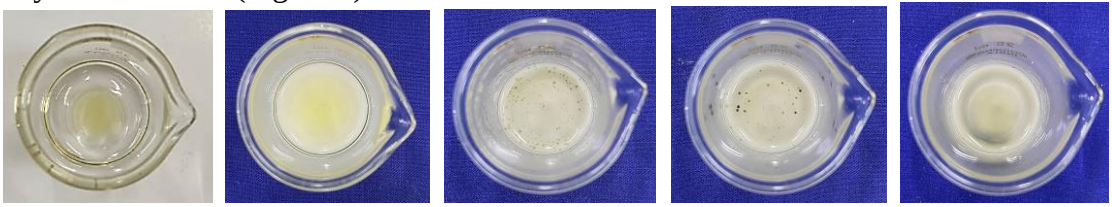
- Lane 1 – olive oil hydrolysis by lipase (1 ml lipase : 2 ml olive oil)
- Lane 2 – olive oil hydrolysis by lipase (2 ml lipase : 2 ml olive oil)
- Lane 3 – Oleic acid standard (Himedia)
- Lane 4 – Oleic acid standard (Commercial)

Figure 4. TLC separation of olive oil hydrolysis by Lipase
(A) olive oil hydrolysis by lipase from *Aspergillus* sp. (18),
(B) olive oil hydrolysis by lipase from *Aspergillus* sp. (11)

Application Studies

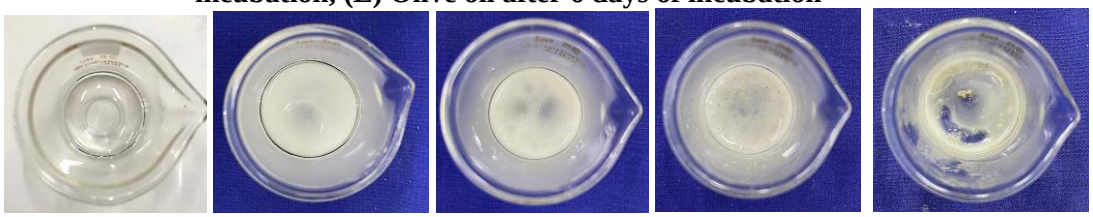
Degradation of lipid

Lipases possess the ability to hydrolyze triglycerides into fatty acids and glycerol. Due to this enzyme ability, crude lipases (enzyme unit of 0.90 U/mL) produced by *Aspergillus* sp. (11) and 2.29 U/mL by *Aspergillus* sp. (18) were applied to degrade olive oil, and coconut oil, which was selected based on the source of lipolytic fungi. Crude lipase produced by *Aspergillus* sp. (11) was able to hydrolyze olive oil and coconut oil effectively after 2 days of incubation (Figures 5 and 6). Crude lipase from *Aspergillus* sp. (18) exhibited the hydrolysis of olive oil on the first day of incubation (Figure 7).



(A) (B) (C) (D) (E) (F)

Figure 5. Observation of hydrolysis activity for olive oil with crude lipase from *Aspergillus* sp. (11)
(A) Olive oil and DW at (0) hour (Control), (B) Olive oil and lipase at (0) hour, (C) Olive oil after 2 days of incubation, (D) Olive oil after 3 days of incubation, (E) Olive oil after 6 days of incubation



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

Figure 6. Observation of hydrolysis activity for coconut oil with crude lipase from *Aspergillus* sp. (11)

(A) Coconut oil and DW at (0) hour (Control), (B) Coconut oil and lipase at (0) hour, (C) Coconut oil after 1 day of incubation, (D) Coconut oil after 2 days of incubation, (E) Coconut oil after 6 days of incubation

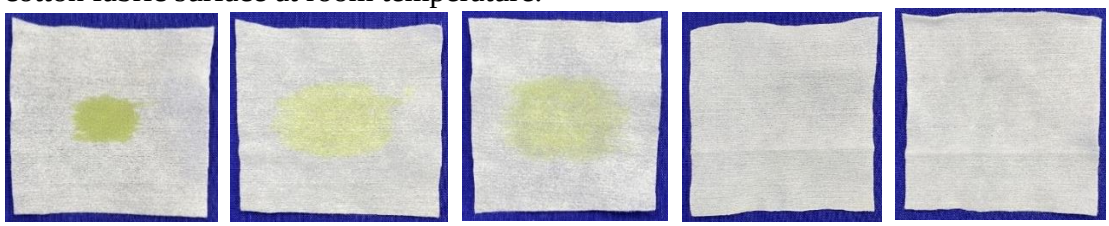
(A) (B) (C) (D) (E)

Figure 7. Observation of hydrolysis activity for olive oil with crude lipase from *Aspergillus* sp. (18)
(A) Olive oil and DW at (0) hour (Control), (B) Olive oil and lipase at (0) hour, (C) Olive oil after 1 day of incubation, (D) Olive oil after 2 days of incubation, (E) Olive oil after 3 days of incubation

Removing oil stains

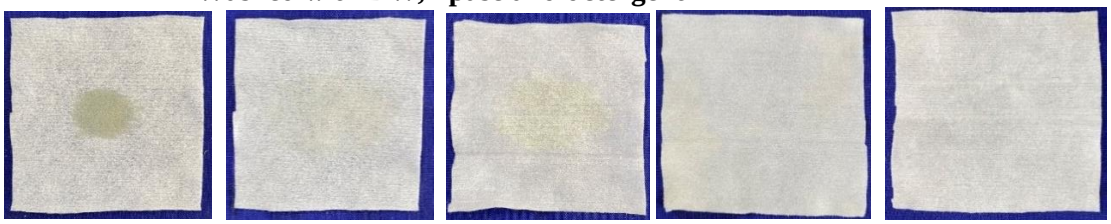
The efficacy of lipase in removing oil (olive oil, fried oil, and vehicle oil) stains was determined by using four treatments, and results were shown in Figure 8 to 10. As laundry additive lipases, partially purified lipase (enzyme unit of 19.52 U/mL) by *Aspergillus* sp. (11), and 1.34 U/mL by *Aspergillus* sp. (18) were applied for removing oil stain on fabric.

The washing performance of partially purified lipase from *Aspergillus* sp. (11) containing detergent greatly improved the cleaning of oil from fabric within 30 min at room temperature as compared to control (Figures 8, 9, and 10). Lipase produced by *Aspergillus* sp. (18) showed the removal of oil stain from fabric when washing was performed with distilled water, detergent and lipase (data not shown). In washing performance, partially purified lipase produced by *Aspergillus* sp. (11) was able to remove a variety of oil stains such as olive oil, fried oil, and vehicle oil from the cotton fabric surface at room temperature.



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

Figure 8. Application of partially purified lipase as a laundry additive lipase from *Aspergillus* sp. (11) in removing fried oil stain after 30 min
(A) Fabric stained with fried oil, (B) Washed with Distilled water, (C) Washed with DW and lipase, (D) Washed with DW and detergent, (E) Washed with DW, lipase and detergent



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

Figure 9. Application of partially purified lipase as a laundry additive lipase from *Aspergillus* sp. (11) in removing olive oil stain after 30 min

(A) Fabric stained with olive oil, (B) Washed with Distilled water, (C) Washed with DW and lipase, (D) Washed with DW and detergent, (E) Washed with DW, lipase and detergent

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

Figure 10. Application of partially purified lipase as a laundry additive lipase from *Aspergillus* sp. (11) in removing vehicle oil stain after 30 min
(A) Fabric stained with vehicle oil, (B) Washed with Distilled water, (C) Washed with DW and lipase, (D) Washed with DW and detergent, (E) Washed with DW, lipase and detergent

Discussion and Conclusion

In the present study, eleven lipolytic fungi were isolated from two different sources such as scraped coconut shell and fuel oil-contaminated soil. Fuel oil-contaminated soil sample was collected from the ground soil of car workshop, Thuwana Township, Yangon Region, Myanmar. Out of 11 lipolytic fungi, two lipolytic fungi based on large clear zones on tributyrin agar medium were selected from two different sources.

The crude lipases produced by two different types of *Aspergillus* species were partially purified by using 30-90% ammonium sulfate precipitation followed by dialysis. Partial purification of lipases from *Aspergillus* spp. (11) and (18) was achieved with 70% ammonium sulfate fractionation and dialysis. Among 2 different *Aspergillus* species, partially purified lipase from *Aspergillus* sp. (11) showed the highest specific activity (2.30 U/mg), which increased 47.82-fold from crude lipase.

In this study, the hydrolysis activity of lipase was investigated by spectrophotometric determination at 715 nm and TLC methods using olive oil as substrate. According to the results of TLC method, it may be concluded that lipases produced by *Aspergillus* spp. (11 and 18) were non-specific lipases (random-specific lipase). Similarly, Rajan *et al.* (2011) stated that lipase produced by *Aspergillus fumigatus* was a nonspecific enzyme.

In the present study, the activity of lipid hydrolysis by crude lipases from *Aspergillus* spp. (11) and (18) was investigated at room temperature. Lipid hydrolysis by crude lipase from *Aspergillus* sp. (11) and *Aspergillus* sp. (18) showed a great hydrolysis capacity in oil degradation. Chuks *et al.* (2008) stated that lipases from *Aspergillus*, *Candida,* and *Fusarium* isolated from crude oil-contaminated soil were potential indicators for microbial degradation of crude oil.

In this study, the cleaning efficacy of partially purified lipases from *Aspergillus* spp. (11) and (18) exhibited a favorable hydrolytic effect on removing oil stains from polycotton fabric. According to the results, lipases produced by *Aspergillus* spp. (11) and (18) effectively removed oil stains from the fabric while distilled water only showed almost no effect on the stained fabric. Partially purified lipase from *Aspergillus* sp. (11) and *Aspergillus* sp. (18) possessed a promising ability to remove oil stain from fabric. Prazeres *et al.* (2006) confirmed that lipase produced by *Fusarium oxysporum* improved the cleaning effectiveness with various commercial detergents. Sharma *et al.* (2017) stated that lipase enzyme containing detergent improved the fabric quality and keeping color bright.

In the present investigation, *Aspergillus* sp. (11) isolated from scraped coconut shell showed the highest lipase activity compared to *Aspergillus* sp. (18) isolated from fuel oil-contaminated soil. Therefore, *Aspergillus* sp. (11) could be used in the industrial sector because of its potential high lipase activities.

In conclusion, the potential of lipase enzyme for detergent industry was investigated. Partially purified lipases produced by *Aspergillus* spp. (11) and (18) had been observed that they had good efficiency in oil hydrolysis and favorable interaction with detergent when lipases were combined with solid detergent. Therefore, lipases from this research may have potential to be used in detergent industry because it was effective in removing oils from oil contaminated fabrics.

The present study was carried out focusing on isolation of lipolytic fungi from different sources, optimization of lipase production, partial purification, and investigation of their applications. Although there have been many reports on the purification and characterization of lipases from fungus in order to apply in industries, fungal lipases from different sources have not been extensively studied in Myanmar.

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