Screening on Lipase-producing Bacteria Isolated from Oil Mill Soil

Khaing Phyo Wai, Weine Nway Nway Oo, and Moe Thazin Shwe

***Abstract*— The present investigation focuses on the attempt of obtaining potential lipase-producing bacteria from oil mill soil. Primary screening of lipolytic activity on agar plates was done with certain substrates such as tributyrin, tween 80 and clear zones and/or opaques around the colonies indicated this activity. Totally 28 lipolytic strains were isolated from soil samples of sesame oil mills located in Mandalay and Myittha Township, Myanmar. They were further evaluated for their qualitative lipase activity by using olive oil as sole specific substrate. Among of them, four strains can be defined as the best lipase producers.**

***Keywords*— Lipase-producing bacteria, oil mill soil, olive oil tributyrin, tween 80**

I. Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis and synthesis of esters formed from glycerol and long-chain fatty acids. They can be used in esterification, interesterification, and transesterification reactions in nonaqueous media with high chemo-, regio- and/or enantio-selectivity. They have emerged as key enzymes which find usage in food, dairy, paper, textile, leather and detergent industries, waste water treatment, production of fine chemicals, pharmaceuticals and cosmetics, synthesis of surfactants and polymers, vegetable fermentation and meat product curing [1-6] . Lipases occur widely in nature, but only microbial lipases are commercially significant [7]. Microbial lipases are more widely applied in industries due to their shorter generation time; ease of bulk production which is further enhanced with advancement in fermentation technologies; and ease of manipulation, either genetically or environmentally. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, diaries, soil contaminated with oil, etc [8]. Many microorganisms such as bacteria, yeast and fungi are known to secrete lipases. Of all these, bacterial lipases are more economical and stable [9]. Bacterial lipases are used extensively in food and dairy industry for the hydrolysis of milk fat, cheese ripening, flavor enhancement and lipolysis of butter fat. Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors, such as temperature, pH, carbon and nitrogen sources, inorganic salts, agitation and dissolved oxygen concentration [10, 11]. In the present study, the indigenous microbes from soil samples of oil processing mill were isolated based on their lipolytic activities.

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II. Materials and Methods

A. Sample Collection

Soil samples from oil mills in Myittha and Mandalay Regions were collected from a depth of 5-10 cm with the help of a sterile spatula and stored in sterile plastic bags. Then, the soil samples were transferred to the laboratory under sterile conditions.

B. Primary Screening of Lipolytic Bacteria

The lipase-producing bacteria were isolated from 1 g of soil sample by serial dilution and spread plate methods on the modified agar medium consisted of Tributyrin (1% v/v), Peptone (0.5% w/v), Yeast Extract (0.3% w/v), Agar (1.5% w/v) at pH 7.0. The plates were incubated at 30°C for 48 hrs. Lipolytic activities of the bacteria were indicated by the formation of clear zones around the colonies. The colonies with Tributyrin-hydrolyzing ability were picked up and streaked on nutrient agar medium as pure cultures.

C. Microscopic Observation

A thin smear of the lipase-producing microbial isolates was made on a clean glass slide which was later stained with Gram's Staining Method. The smear after air drying was observed under the microscope at 10x with immersion oil to study the Gram's staining reaction and morphology.

D. Egg-Yolk Test for Primary Lipolytic Activity

The egg-yolk test medium was made by melting the nutrient agar, cooling to 55°C and adding the egg-yolk suspension. After two days incubation, saturated copper II sulphate ($CuSO_4$) solution was poured into the plate, and stood for 20 minutes. Then, the excess solution was drained off and the plates were dried for a short time in the incubator. The greenish-blue colour of copper soaps of fatty acids was confirmed lipolysis.

E. Screening of the Lipase-producing Bacteria

Another method for detecting lipolytic activity in microorganisms was the use of the surfactant Tween 80 in a solid medium. Isolates were grown on Tween 80 medium and incubated at 30°C for three days. The formation of opaque zones around the colonies was an indication of lipase

production by the isolates. Also, the production of lipase activity was verified on Olive Oil agar medium containing 1% high refined olive oil (v/v) and fluorescent dye Rhodamine-B (0.005 %)(dissolved in distilled water and sterilized by filtration). Orange-coloured fluorescent halos around lipase-producing colonies were seen when these agar plates were exposed to UV light 350 nm.

III. Results and Discussions

A. Primary Screening of Lipolytic Bacteria

Isolation using Tributyrin agar plates shows clear zones around the colonies and indicates the lipolytic activity (Fig.1). An emulsion of micro-droplets of the fat, Tributyrin, in a solid medium makes it opaque and lipolytic organisms remove the opacity by converting the fat to water-soluble butyric acid. Totally 28 strains of bacteria (L1-L28) were isolated. The bacterial cultures were recognized according to the cell morphology and gram staining reaction.

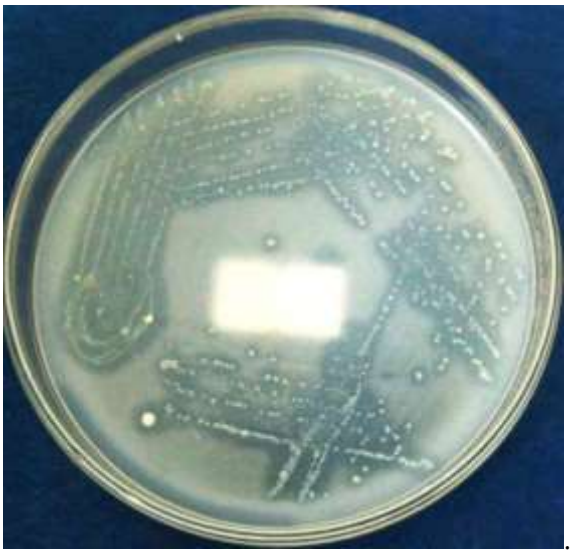

Fig.1 Lipolytic activity on 1% tributyrin agar medium

B. Egg-yolk screening test for Primary Lipolytic Activity

The egg-yolk suspension allows for the detection of lecithinase and lipase activity of the bacteria. On solid media containing egg-yolk, lipolysis is shown by the formation of a thin, iridescent 'pearly layer' overlying the colonies and a 'confined' opalescence in the medium underlying them, seen best when the colonies are scraped off. Copper sulphate can be used to form bright greenish-blue insoluble copper soaps with the fatty acids in both the pearly layer and opalescence in the medium. Lipase destroys the fats within the egg yolk, which results in greenish blue colour of the colony surface when flooded with copper II sulphate solution (Fig.2).

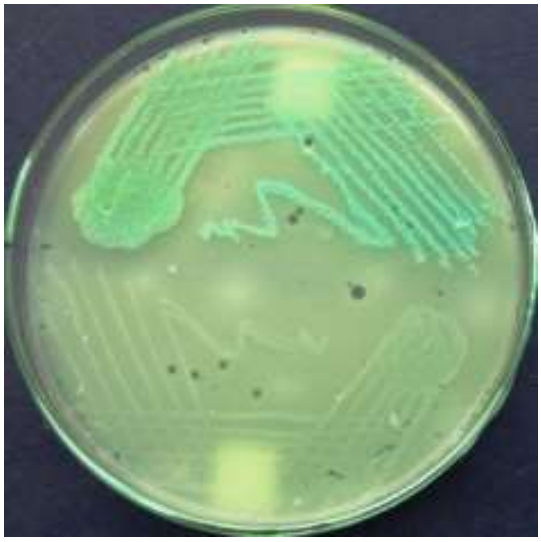

Fig.2 Egg-yolk test of isolates

C. Screening for Lipolytic Activity

Tweens (fatty acid esters of polyoxyethylene sorbitan) have been the most widely used substrates for the detection of lipase/esterase producing microorganisms in agar media [12]. Screening using tween agar plates shows precipitation around the lipase/esterase producing micro-organisms. The method is based on the precipitation as the calcium salt of the fatty acids released by hydrolysis of tweens. Liberated fatty acids bind with the calcium incorporated into the medium. The calcium complex is visible as insoluble crystals around the inoculation site.

Tween 80 is mostly hydrolyzed by the lipase as it contains esters of oleic acid and rarely by esterases while tween 20 is easily hydrolyzed by esterase as it contains esters of lower chain fatty acids viz. lauric acid. In the present study, formation of precipitation on Tween 80 agar plates by the isolates also confirmed that this bacterial strain produced lipase (Fig.3). One of the disadvantages of using Tributyrin and Tween 80 is that they can be hydrolyzed by the esterases giving false positive results for lipase presence.

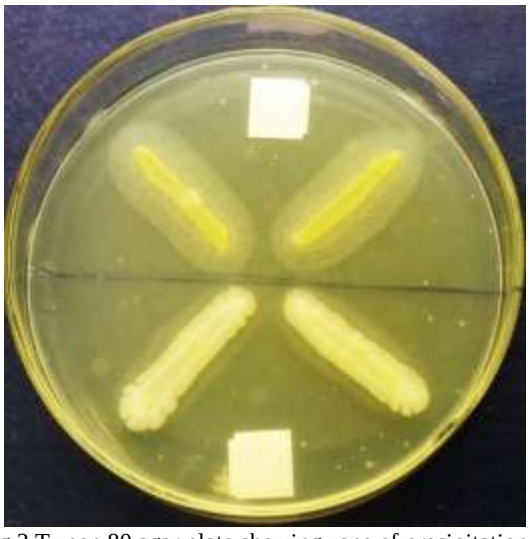

Fig.3 Tween 80 agar plate showing zone of precipitation

D. Screening of True Lipase Activity

True lipase activities of the isolates were verified on Rhodamine B olive oil agar plates at both 25°C and 4°C. Orange-coloured fluorescence halos around lipase producing colonies were seen when these agar plates were exposed to UV light at 350 nm. While bacteria gave positive result to lipase activity after three days at 25°C, orange colour observed after a week at 4°C (Fig.4). Rhodamine B dye test gave more convenient results than others because the test showed positive results to only lipase existence and not affected by bacterial metabolite wastes. This method is not sensitive to the pH changes and does not inhibit the growth of bacteria. Out of these, four bacterial isolates were shown lipase activity.

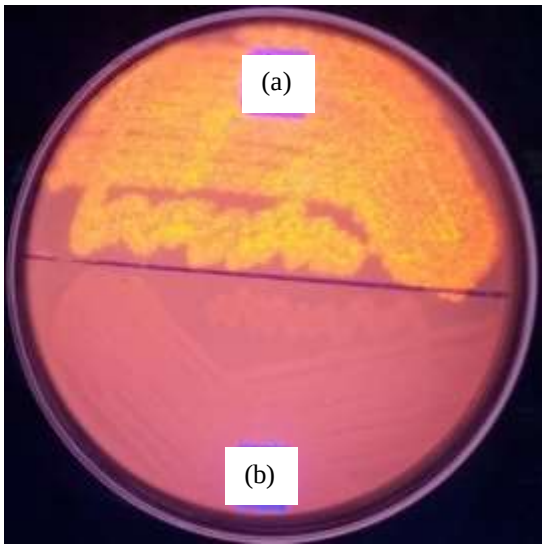

Fig.4 Lipase activity of the isolates on olive oil + rhodamine B agar plate (a) Lipase-positive bacteria, (b) Lipase-negative bacteria

IV. Conclusions

The present study confirms that the bacterial strains L3, L14, L20 and L25 have the lipase and esterase activities. Therefore, they can be further studied as the locally isolated strains for industrial applications. The taxonomy of the isolated strains can be further examined by using Bergey's Manual of Determinative Biology and confirmed by 16S rDNA sequencing. The optimization studies can also be performed with other physiological parameters for their possible exploration at lab and industrial scale production of lipase.

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