ISOLATION, IDENTIFICATION AND SCREENIG OF LIPASE ACTIVITY OF BACTERIAL ISOLATE

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ABSTRACT

The dominant bacterial isolate was isolated from soil near active mud volcanoes (Minbu).Isolated strain was identified by microscopy, colony morphology a, biochemical characters and 16 s RNA sequences aligning sequences from NCBI database . According to these analysis, the isolated strain was Bacillus oceanisediminis and it has lipase enzyme on 1 % of the four oil; almond oil, groundnut oil, olive oil and sesame oil. Methyl red reagent was used for difference of clear zone. Although lipase producing activity on the four oil based media was not very different of lipase producing activity, olive oil and groundnut oil added culture media were more clear zone around bacterial streak. Some enzyme activity was tested and protease activity on litmus milk media was also observed. Bacillus oceanisediminis strain gave pink color of positive result. Growth condition of pH, temperature, and NaCl usage were broad range. In this study, dominant strains of volcanic environment was characterized and the potential activity of isolates were observed and can be used to industrial purposes, food industries and many other applications

Keyword: - *Bacillus oceanisediminis, lipase, 16 s RNA sequence ,olive oil, groundnut oil, sesame oil, almond 0il*

1. INTRODUCTION

Microbial lipases are one of the important groups of industrially and commercially produced enzymes. Compared to plant and fungal lipases, a relatively small number of bacterial lipases have been well studied and reviewed [1] [2]. Generally, bacterial lipases are glycoproteins but some extracellular bacterial lipases are lipoproteins. The production of extracellular lipases from bacteria is often dependent on nitrogen and carbon sources, inorganic salts, presence of lipids, temperature and availability of oxygen. It was reported in 1979 [3].

Most bacterial lipases are non-specific in substrate specificity and a few are thermostable [4]. Different genera of bacteria including *Streptomyces* spp. produce lipase but the following genera have been well exploited for lipase production : *Achromobacter* spp., *Alcaligenes* spp., *Arthrobacter* spp., *Pseudomonas* spp. and *Chromobacterium* spp [5]. Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) catalyze the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Lipases occur widely in nature, but only microbial lipases are commercially significant. The many applications of lipases include specialty organic syntheses, hydrolysis of fats and oils, modification of fats, flavor enhancement in food processing, resolution of racemic mixtures, and chemical analyses. This article discusses the production, recovery, and use of microbial lipases. Issues of enzyme kinetics, thermostability, and bioactivity are addressed and also productions of recombinant lipases are detailed. Immobilized preparations of lipases are discussed. In view of the increasing understanding of lipases and their many applications in high-value syntheses and as bulk enzymes, these enzymes are having an increasing impact on bioprocessing.

Commercially useful lipases are usually obtained from microorganisms that produce a wide variety of extracellular lipases. Many lipases are active in organic solvents where they catalyze a number of useful reactions including

esterification [6-12] transesterification, region selective acylation of glycols and menthols, and synthesis of peptides [13] and other chemicals [14-16].

2. MATERIALS AND METHODS

2.1 Isolation of Bacteria Strains

Bacterial strains were isolated from sediment near mud volcanoes in Minbu township, Myanmar. Dominant colonies were sub cultured and sub cultured to Nutrient medium for purified single colonies.

2.2 Determination of Cultural and Microscopic Morphologies

Colony morphology of isolated strains was observed on 24 hours incubation culture of nutrient medium. For microscopic morphology, bacterial smear under microscope was examined after Gram stain reaction.

2.3 Biochemical characteristic of bacterial isolates Determination of Cultural and Microscopic Morphologies

Some biochemical tests have been performed on bacterial isolates for classifying them according to their biochemical features. The tests include Oxidase test, Catalase test, litmus milk test, starch hydrolysis test, Motility test, by standard methods.

2.3 Analysis of 16S rRNA sequencing

Sequencing of RNA was performed by 1500 bp PCR product. For the sequence analysis ABI automated sequencer was used. The 16S rRNA sequences were aligned and compared with other 16S rRNA genes in the GenBank by using the NCBI Basic Local Alignment Search Tools (BLAST).

2.4 Screening on Lipase Producing Bacteria

For qualitative examination, different substrates; 1% of almond oil, groundnut oil, olive oil and sesame oil were used. Each of these substrates and methyl red indicator (0.1 %)were added to nutrient media (nutrient broth of Hi media 13g/L and 20 g agar /L) at 50ċ- 60 ċ. These plates were incubated at 37 ċ for 24 to 48 hours. Colonies showing the zone of lipolysis around them were selected and were further purified on selective agar plate.

3. RESULTS AND DISCUSSIONS

Cultural and microscopic morphologies of isolated strain was shown in figure1and 2. Some biochemical characters of bacteria was shown in table 1and 2. These bacterial isolates were characterized on the basis of colony characteristics, microscopic appearance and biochemical tests in figure 3. Molecular characterization of these strains was done by 16S rRNA analysis.

According to these tests, the isolated dominant bacteria is *Bacillus oceanisediminis* strain C8_CDM2 16S ribosomal RNA gene, partial sequence and accession number is MK850252.1 on NCBI blast. *Enzyme activities were tested and positive protease activity on litmus milk media was observed. B. oceanisediminis* strain gave pink color of positive result. It gave negative result on starch hydrolysis. Growth condition of pH, temperature, and NaCl usage were broad range.

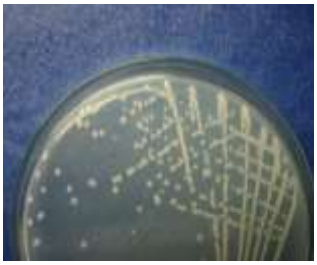

Fig -1: **Cultural morphology of *Bacillus oceanisediminis***

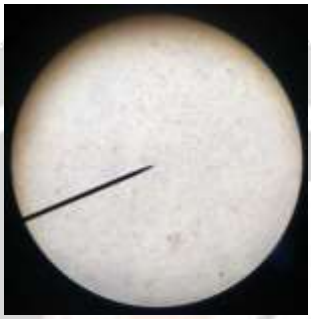

Fig -2: Microscopic morphology of *Bacillus oceanisediminis*

Fig -3: Litmus milk test

Table1: Biochemical Characteristics of Bacterial Isolate M1

Strain	Colony shape	Colony color	Gram reaction	Cell size	Catalase test	Oxidase test	Litmus milk test	Starch hydrolysis	Motility
M1	circular	Light yellow	positive	0.5-1um , 1.5-2.5um	+	+	+	-	+

+ = positive result, - = negative result

Table2: Growth condition of Bacterial Isolate M1

Strain	Growth on NaCl	Temperatre	pH
M1	3-7%	20 - 40ċ	6 -8

Bacillus oceanisediminis strain gave clear zone formation around bacterial streaks of 1% oil each. These are shown in figure 4. Therefore, B. *oceanisediminis gave* lipase producing activity. In this study, different oils were used, lipolytic activity was not very different. But activities on groundnut oil and olive oil were more clear zone of B. *oceanisediminis*

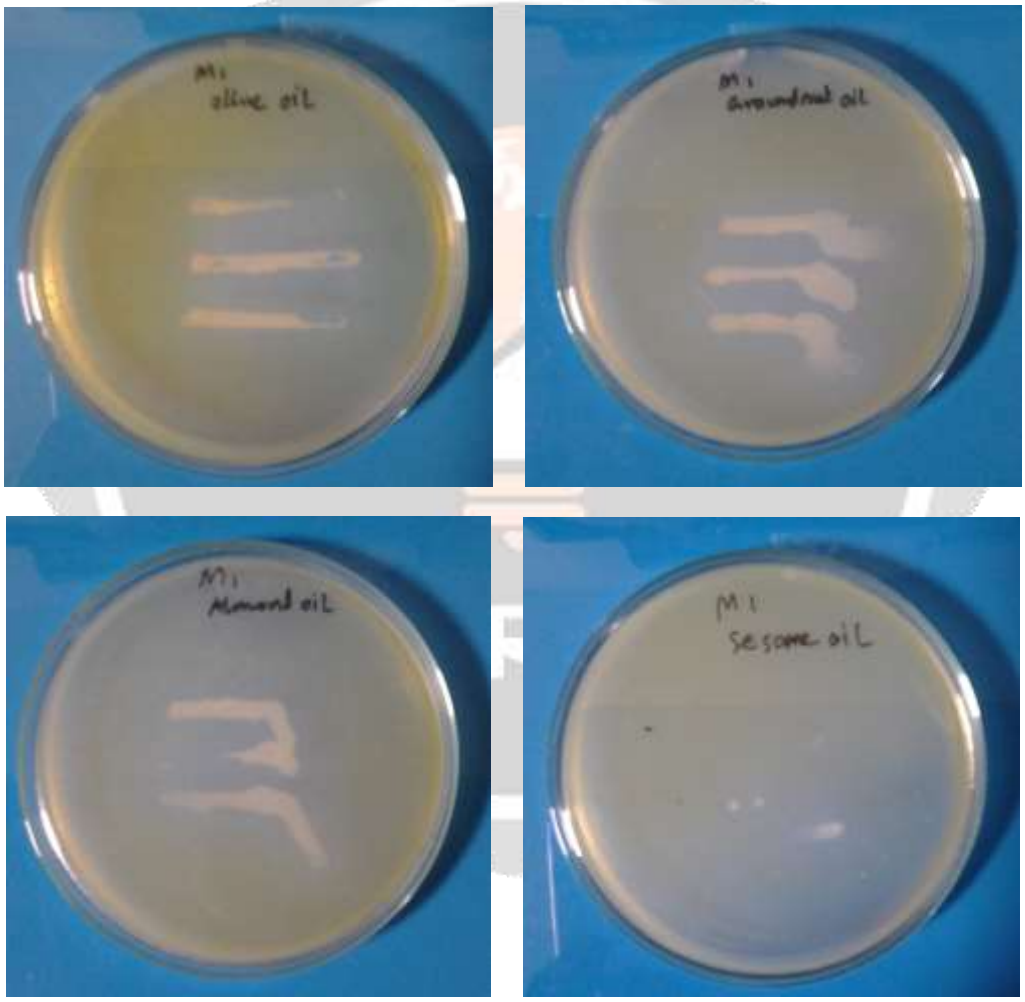

Fig -4: Lipase producing activities of *Bacillus oceanisediminis*

4. CONCLUSIONS

Bacillus oceanisediminis strain was isolated from sediment of active volcano and identified by morphologies, biochemical characters and molecular characterization of 16 RNA sequencing analysis using NCBI database alignments. Some enzyme activities were observed such as protease (litmus milk test), lipase producing activity. Therefore, the strain is potential for industrial application and others activities should be investigated.

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