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LIPASE PRODUCTION FROM AGRICULTURE WEED *Argemone mexicana* SEED OIL BY NEWLY ISOLATED MUTANT BACTERIAL STRAIN UNDER OPTIMIZED NUTRITIONAL CONDITIONS

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ABSTRACT

The goal of the present investigation was to isolate *Bacillus pumilus* KMAS7, a lipase producing bacteria, from edible oil contaminated soil of Cheyyar. The identity of the organism was established by 16S rRNA sequence, in addition to the morphological and biochemical characterization. A mutant strain was developed by random mutation using UV-C radiation. Following mutagenesis and screening, a mutant designated *B. pumilus* UVM was selected due to its high lipase activity. The selected mutant strain was cultivated in submerged fermentation for lipase production using agriculture weed *Argemone mexicana* seed oil under different nutritional sources. The maximum lipase of 62.48±0.05 UmL^{-1} was obtained in the submerged fermentation using 0.5% w/v glucose, 1% w/v ammonium sulphate and 10 μML^{-1} Ni as carbon, nitrogen and trace element sources respectively. Though *A. mexicana* seed oil has been used for biodiesel production, to our knowledge this is the first report on the lipase production by newly isolated mutant bacterial strain.

Keywords: ***Argemone mexicana, Bacillus pumilus*, lipase, submerged fermentation, mutation**

INTRODUCTION

Lipases (EC.3.1.1.3, triglyceride-based hydrolases) are a group of enzymes with many uses in biotechnological applications, including the ability to catalyze the hydrolysis of fats to fatty acids and glycerol at the oil-water interface. Also, catalyze the opposite reactions in water-free medium for ester synthesis and transesterification **[1]**. They belong to the class of serine hydrolases and therefore do not require cofactors. Chemically, regio- and enantiomerically specific behavior of lipases has attracted the attention of researchers and industrialists **[2]**. In the future, lipases will dominate the global enzyme market as they are the most important group of biocatalysts in new biotechnological applications **[3]**. Various species of bacteria, fungi, animals and plants have been reported by many studies to be the main sources of lipases. However, microbial lipases are used in different applications in many industries including food, dairy, paper, textile, leather, pharmaceutical, fine chemicals, detergents, wastewater treatment, and biodiesel production because they are stable at higher temperatures, organic solvents, detergents, regiospecificity, stereospecificity, broad substrate specificity, and the ability to catalyze heterogeneous reactions at the interface of water-soluble and water-insoluble systems **[4-6]**. Recently, a new method of cleaning septic tanks, grease traps and other systems using lipases has been investigated in environmental research **[7]**.

Microorganisms like bacteria and fungi have the ability to produce intracellular and extracellular lipases. Lipases of microbial origin, especially bacterial lipases, have been widely used in biotechnological applications since their discovery in 1856 **[4, 8]** due to their stability, selectivity, and wide substrate specificity. Lipase-producing bacteria are usually isolated from environments where lipids and conditions appropriate for lipase production are present. The isolated bacteria are evaluated for their ability to produce lipase in solids or liquids media and the enzymes are characterized and purified **[9]**. Among various bacteria, *Bacillus* spp., especially *B. subtilis* and *B. pumilus* for lipase production in many industry is generally customary because they able to produce either alkaline or thermophilic lipases at extreme culture conditions.

The marketplace value of microbial lipases was estimated at $425 million in 2018 and is expected to reach $590.2 million by 2023 **[3]**. For commercial applications, lipases need to be produced on a large scale by fermentation. Fermentation technology is a process in which bacteria grow and metabolize complex substrates, breaking them down into simpler compounds and producing more enzymes and byproducts as they do so. Since microbial lipase is mostly extracellular, it can be produced via solid-state fermentation (SSF) or submerged fermentation (SmF) **[10]**. One of the disadvantages of commercial lipase enzyme production is the cost of production resulting from expensive carbon and nitrogen inputs, which account for 50% of the total cost of enzyme production. The use of low-cost raw materials becomes important when developing the production of microbially derived lipase **[11, 12]**.

The current aim is to develop new methods for the production of commercially important enzymes to make the entire process commercially profitable **[13]**. Exploration and quantification of new residues is considered a useful method when it facilitates the production of enzymes of microbial origin and reduces production costs and problems associated with waste disposal **[11, 14]**. Most bacterial lipases reported to date are constitutive and nonspecific in their substrate specificity, and a few bacterial lipases are thermostable. The fermentation physico-chemical parameters like pH, temperature, size of inoculum, stirring rate, inducer sources, carbon sources and concentration are affected the production of extracellular lipases by bacteria **[15, 16]**.

The huge demand for lipase has forced researchers to find new strains that can be grown in cheaper media. In general, vegetable oils are used as inducers for lipase production, in addition to being carbon source. This article focuses on high lipase production using low-cost raw materials under suitable conditions. To date, no attempts have been made to produce lipase from agriculture weed *A. mexicana* seed oil. Therefore, the aim of this study was focused on lipase production from *A. mexicana* seed oil by newly isolated mutant strains under optimized nutritional conditions. *A. mexicana* is an annual plant found as a weed plant worldwide. Plant height is 0.5-1.5 m and matures in 110-120 days. It should rain in temperate and tropical regions. The plant loves all types of soil. The seeds are black and heavy. The seeds contain approximately 30% oil and their fatty acid composition includes 25% oleic acid and 55% linoleic acid **[17]**.

MATERIALS AND METHODS

All chemicals used in the experiment were purchased with high purity or analytical grade from Hi-media and SRL (Mumbai, India) for preparing various media and reagents.

Soil sample collection and isolation of lipase producing bacterial strains

Soil samples were collected aseptically from five different sites, which are far from each other, viz. I, II, III, IV, and V of edible oil spilled areas of the small-scale oil extracting industry, Cheyyar Taluk, Tiruvannamalai District, Tamil Nadu, India, for the isolation of potent lipase producing bacterial strains under laboratory conditions. The obtained soil samples were pooled into a single sample and used 1 g of pooled soil sample for soil extracts by dissolving in 100 mL distilled water. Then the sample was serially diluted by added about 1 mL of sample into 9 mL sterilized distilled water. This is the first dilution, and it gives a 10^{-1} dilution. Like this, up to 10^{-9} dilutions were made. Finally, the dilutions of 10^{-3} to 10^{-5} were chosen and spread 100 µL of diluted sample on a nutrient agar plates supplemented with olive oil (1% v/v). Then plates were incubated at 30 ºC until the strains with lipolytic activity.

Maintenance of isolated bacterial strains

Isolated bacterial strains were stored at 4 °C in a nutrient agar slant (5.0 g Peptone, 3.0 g Beef extract, 5.00 g Sodium Chloride, 15.0 g Agar, 1000 mL Distilled water, pH 7.1± 0.2) for further uses. The cultures were revived every month. An inoculum was prepared by transferring a loopful of stock culture to the nutrient medium. The inoculum culture was cultivated at 35 °C until an optical density (OD) value of 0.6 was reached at 600 nm.

Plant material

Serious agricultural weed *Argemone mexicana* L., seed oil was used as an inducer for lipase enzyme production using newly isolated bacterial strains. This plant material was authenticated in PG & Research Department of Botany, Arignar Anna Government Arts College, Cheyyar - 604 407, Tamil Nadu, India. A herbarium of this plant was prepared and deposited in the Department for reference.

Seeds collection from *A. mexicana*

Matured fruits of *A. mexicana* were collected from natural habitat of Cheyyar (Tiruvetipuram) Taluk in the Tiruvannamali District, Tamil Nadu, India in middle of March 2021. It has geocoordinate of latitude 12.658° N and longitude 79.5434°E. Each fruit covered with thorns and contained many small mustard like seeds. The seeds were separated from fruits along with tiny thorns. Thorns were removed from seeds using conventional techniques and then the seeds were dried at sun light for 8 h. The dried seeds were used for oil extraction.

Oil extraction from *A. mexicana* seeds

Prior to the oil extraction, the excess moisture of seeds was removed by again dried in the hot air oven at 60 ºC for 24 h. The dried seeds were weighed and ground into a powder. Then it was used for oil extraction by Soxhlet extraction using solvent n-hexane with the ratio 5:1 (5L solvent:1kg seeds). Oil extraction was performed until the complete extraction from seed powder. Finally, the oil was recovered from the solvent using rotary evaporator. The mass percentage of oil extracted from seeds was calculated using the following formula.

$$\text{Mass \%} = \frac{\text{Weight of the oil}}{\text{Weight of the oil seed}} \times 100$$

Screening of maximum lipase producing strain

Erlenmeyer flasks (250 mL) containing 100 mL of basal medium (3 g Yeast extract, 1.5 g K_2HPO_4, 1.5 g KH_2PO_4, 0.3 g CaCl, 0.4 g $MgSO_4.7H_2O$, 0.1 g KCl, 0.3 g $FeSO_4.7H_2O$, 1000 mL Distilled water, pH 7.0) was supplemented with 1.0 % (v/v) of *A. mexicana* seed oil and inoculated 1% (v/v, OD 0.92) of newly isolated bacterial strains. Olive oil (1%, v/v) contained medium was used as control for each isolate. Then the Erlenmeyer flasks were incubated at 35 °C for 48 h. End of the experiment, culture broth was withdrawn and centrifuged at 14,000 rpm for 15 min using a centrifuge (MPW-352/R/RH Centrifuge, MPW MED Instruments, Poland). A standard assay was performed to determine the lipase activity of cell free supernatant and selected the maximum lipase producing bacterial strain for further investigation.

Lipase assay

Extracellular lipase activity was assayed spectrophotometrically **[18]**. The *p*-nitrophenyl palmitate (*p*-NPP) was used as a substrate. The mixture of 20 µL of substrate solution, 200 µL of crude lipase and 1.8 mL of Tris-HCl buffer (0.1M, pH 8.0) was incubated at 37 ºC for 10 min. Finally, the reaction was terminated by the addition of 2 mL of 0.2 M Na_2CO_3 solution. Released *p*-nitrophenol (*p*-NP) was immediately determined by measuring the absorbance at 400nm in a UV-Visible spectrophotometer (Shimadzu UV-1601, Mumbai, India). The OD values were interpolated in the standard curve of *p*-nitrophenol and found the unknown concentration of *p*-nitrophenol. One unit (IU) of lipase activity was defined as micromole(s) of *p*-nitrophenol released by hydrolysis of *p*-nitrophenyl ester by one milliliter of soluble enzyme per minute at 37°C under assay conditions.

Cellular morphology and biochemical characterization of strain KMAS7

The selected strain KMAS7 was identified based on morphological observation and biochemical characterization. Morphological characteristics viz. colony shape, colony elevation, colony margin, cell shape, endospore, motility and Grams staining and biochemical tests (catalase test, gelatin hydrolysis, indole test, voges-proskauer test, oxidase test, starch hydrolysis test, urease test, citrate test, triple sugar iron, dextrose fermentation test and sucrose fermentation test) were carried out according to Bergey's Manual of Determinative Bacteriology **[19]**.

Molecular level identification of strain KMAS7 using 16S rRNA based techniques

Genomic DNA was extracted from the selected strain KMAS7 using the Biogeno Genomic DNA isolation kit (BGKT16). The amplification of 16S rRNA gene was carried out by polymerase chain reaction (PCR) using primer (F) 5'-AGAGTTTGATCCTGGCTCAG-3' as the forward primer and (R) 5' - TACGGTTACCTTGTTACGACTT-3' as the reverse primer. The PCR and sequencing were performed according to the manufacturer's protocol using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems). Then the sequence of 16S rRNA was aligned by using the BLASTn programme **[20]**, and a Neighbour - Joining phylogenetic tree was constructed using the Clustal W algorithm with the help of MEGA-X (Molecular Evolutionary Genetics Analysis) software version 10.2.2 **[21]**.

Strain development by UV-C mutation

The selected wild parent strain KMAS7 was exposed to UV-C irradiation at varying time intervals of 0, 2, 4, 6, 8, and 10 min in a "Laminar flow hood – Cabinet" fitted with a TUP 40W germicidal lamp that has about 90% of its radiation at 2540-2550A at an affixed distance of 30 cm away from the lamp. After that, the irradiated plates were covered with black cloth and incubated for 24 h at 35 ºC. Plates having less than 1% survival rate was chosen for mutant strains. Totally, eleven mutant colonies were obtained from wild parent strain KMAS7 and they were designated as UVM1-UVM11. All the colonies were screened for lipase production with 1% (v/v) of *A. mexicana* seed oil. Based on the highest lipase production the mutant strain was selected for further lipase production. Every month, the mutant strain was revived in a nutrient agar slant.

Effect of different concentration of *A. mexicana* seed oil on lipase production

The effect of different concentration of *A. mexicana* seed oil range from 1 to 10 % v/v, on lipase production by selected mutant bacterium was studied. This study was carried out in 250 mL Erlenmeyer flask using 100 mL of the production medium amended with different concentration of *A. mexicana* seed oil. Then flasks were incubated at 35 °C for 48 h at 120 rpm using 1% v/v (OD 0.92) inoculum. At the end of the experiment, 10 mL of culture broth was withdrawn and centrifuged at 14,000 rpm for 15 min using a centrifuge (MPW-352/R/RH Centrifuge, MPW MED Instruments, Poland). The cell free supernatant was used as the crude lipase for determination of lipase activity under standard assay conditions. The best concentration of *A. mexicana* seed oil was used for further media optimization.

Screening the best nutritional factor for lipase production by mutant strain using classical method of optimization

The best nutritional factors were screened to achieve the maximum lipase production using a selected mutant strain by the classical method of optimization (one-parameter-at-a-time). The experiments were carried out in a 250 mL Erlenmeyer flask using 100 mL of each sources free basal medium with optimum concentration of *A. mexicana* seed oil as inducer. The medium pH was adjusted into 7 using 1N NaOH or HCl. The medium was autoclaved at 1.1 bar pressure for 15 min at 121°C. The fermentation was then performed at 35 °C for 48 h at 120 rpm using 1% v/v inoculum (OD 0.92). At the end of the experiment, 10 mL of culture broth was withdrawn and centrifuged at 14,000 rpm for 15 min using a centrifuge (MPW-352/R/RH Centrifuge, MPW MED Instruments, Poland). The cell free supernatant was used as the crude lipase for determination of lipase activity under standard assay conditions. The essential nutritional factors such as carbon sources (0.5% of w/v, glucose, fructose, sucrose, maltose and starch), nitrogen sources (1% w/v of beef extract, yeast extract, peptone, ammonium chloride, and ammonium sulphate) and metal ions (10 μML^{-1} of Iron (Fe) as $FeSO_4.7H_2O$, Copper (Cu) as $CuSO_4.7H_2O$, Selenium (Se) as Na_2SeO_3, Nickel (Ni) as $NiSO_4.7H_2O$ and Zinc (Zn) as $ZnSO_4.7H_2O$) were optimized.

Statistical analysis

In this study, data obtained were subjected to statistical analysis to determine means and standard deviations by Software-MINITAB 12. All the experiments were carried out in triplicate, the values presented in the graph are those of the mean of three independent experiments and the error bars indicate standard deviation. Similarly the values presented in the table are those of the mean ± SD of three independent experiments. The standard deviation did not exceed 5% of the average values.

RESULTS AND DISCUSSION

Isolation and screening of lipase producing bacteria

There is an immense need to explore natural habitats to isolate lipase-producing potential bacteria, because microbial isolation and screening is a significant stage for evaluating potential lipase-producing bacteria from different natural habitats. In the present investigation, soil samples were collected from five different places of an edible oil spilled areas of the small-scale oil extracting industry which is located in and around Cheyyar Taluk, Tiruvannamalai, Tamil Nadu, India and utilized as a source for isolation of potential bacterial strain for lipase production from agriculture weed *A. mexicana* seed oil. Totally, seven lipase producing bacterial strains were isolated from soil samples under aerobic conditions. Subsequently each strain was quantitatively screened by cultivated in the basal medium supplemented with *A. mexicana* seed oil (1%, v/v) for 48 hrs. Olive oil (1%, v/v) contained medium was used as control for each isolate. It was observed that all the seven bacterial strains were grown well and produced lipase in *A. mexicana* seed oil (1%, v/v) supplemented basal medium. Beside all the isolates were utilized *A. mexicana* seed oil as an inducer for lipase production. As shown the **Table 1**, only the strain KMAS7 was produced maximum lipase of 27.12±0.04 UmL^{-1} and 25.23±0.15 UmL^{-1} in medium contained 1% (v/v) of *A. mexicana* seed oil and olive oil respectively. However, strain KMAS7 showed 1.99 UmL^{-1} higher lipase production in *A. mexicana* seed oil than the olive oil contained medium. Therefore, the strain KMAS7 was selected for further study. This result supports the fact that lipase production among the bacterial species could be notably different **[22, 23]**. This is the first report on the lipase production by newly isolated bacterial strain using *A. mexicana* seed oil.

Morphological and biochemical characteristics of strain KMAS7

The selected strain KMAS7 colony was round, raised and entire. By the microscopic observation, it was observed as rod shape and Grams positive bacterium. For taxonomic identification, the KMAS7 was subjected to a series of biochemical tests and obtained positive results for catalase test, dextrose fermentation test, sucrose fermentation test, Voges-Proskauer test and oxidase test. Similarly, negative results were obtained for gelatin hydrolysis, indole test, starch hydrolysis test, urease test, citrate test and TSI test **(Table 2)**. These results were compared with existing literature and found that the strain KMAS7 was similar with *Bacillus pumilus*. This result is in accordance with the study of Parvathi *et al*., **[24]**.

Table 1: Quantitative screening of extracellular lipase producing bacteria

Bacterial Culture Code	Lipase activity (UmL^{-1})	
	Control- Olive oil	*Argemone mexicana* seed oil
KMAS1	15.14±0.02	16.15±0.14
KMAS2	12.12±0.11	14.32±0.04
KMAS3	23.32±0.12	23.15±0.14
KMAS4	11.21±0.31	12.24±0.11
KMAS5	19.32±0.14	18.24±0.05
KMAS6	18.15±0.05	19.48±0.13
KMAS7	25.13±0.15	27.12±0.04

(All values are represented as mean±SD of three replicates)

Table 2: Morphological and Biochemical characteristics of isolate KMAS7

Characters	KMAS7
Morphological characteristics	
Colony shape	Round
Colony Elevation	Raised
Colony margin	Entire
Cell shape	Rod
Endospore	+
Motility	Motile
Grams Staining	Positive
Biochemical characteristics	
Catalase test	+
Gelatin Hydrolysis	-
Indole test	-
Voges-Proskauer test	+
Oxidase Test	+
Starch Hydrolysis Test	-
Urease test	-
Citrate test	-
Triple sugar iron (TSI)	-
Dextrose fermentation test	+
Sucrose fermentation test	+
Isolate identified as	*Bacillus pumilus*

+ =positive result; - = negative result

Molecular identification of strain KMAS7

Molecular level identification of the selected strain KMAS7 was done by 16S rRNA sequence analysis. The gene encoding of 16S rRNA was amplified by a PCR technique and it was found that the amplified product size was ~1437 bp. The selected bacterial strain sequence was matched with 26 closely related bacterial sequences in the GenBank database for sequence similarity search. The evolutionary history was inferred using the Neighbor-Joining method **[25]**. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (50 replicates) are shown next to the branches **[26]**. The evolutionary distances were computed using the maximum composite likelihood method **[27]** and are in the units of the number of base substitutions per site. The proportion of sites where at least one unambiguous base is present in at least one sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 26 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 1353 positions in the final dataset. Evolutionary analyses were conducted in MEGA X **[21]**.

It was observed from **Figure 1** that the marked isolate KMAS7 was nearly 100% closed to the strain *Bacillus pumilus* NBRC12092. The data of phylogenetic studies unveiled and conformed that the selected isolate KMAS7 was *B. pumilus*. Consequently, the respective sequence of the isolate was submitted to GenBank as *Bacillus pumilus* strain KMAS, and the allotted GenBank accession number was OR264494. Then it was used for further investigation. *Bacillus* species constitute a diverse group of bacteria widely distributed in soil and the aquatic environment **[24]**. Various *Bacillus* species have also been found in a variety of environmental niches **[28, 7]**. There are many documents in the literature describing 16s RNA as the best analytical method for identifying various bacterial strains from different environmental samples **[23, 29-31]**.

Selection of mutants with high lipase production

Significant increases in fermentation efficiency and cost reductions were achieved with mutagenesis. In this study, suspensions of selected wild parent strain *B. pumilus* KMAS was exposed to UV-C radiation for 3, 6, 9, 12, 15, and 18 min. The UV-C survival percentage at each exposure time was tabulated **(Table 3)**. The mutation frequency was mentioned to be high when the survival rates were between 10 and 0.1% **[32]**. Plates having less than 1% survival rate of the parent strain were selected. A total of eleven mutant strains were obtained and they are labeled as UVM1 to UVM11. Subsequently, all the eleven strains were screened for maximum lipase production. As shown in **Table 4**, the mutant strain UVM5 was produced maximum lipase of 34.15 ± 0.04 UML^{-1}. It was 1.26 times more abundant than the wild parent strain KMAS7 (27.12 ± 0.12 Uml^{-1}), thus it was designated as a mutant strain and the name *B. pumilus* UVM was added. This strain was used for further study. Lakshmi and Dhandayuthapani **[23]** also conducted a similar study. Vijayabaskar *et al* **[33]** reported a UV mutant type *B. pumilus* UVR10 produced 1.330 ± 0.075 $IUmL^{-1}$ carboxymethyl cellulase. Mutation breeding has become a better method for bacterial strain development than genetic engineering. Inducing ultraviolet mutation is the easiest and most effective way to make the random mutation in the bacterial strains. The characteristic of the ultraviolet wavelength is around 255 nm, which is the same as the DNA absorption spectrum of general bacteria **[34]**.

Effect of different concentration of *A. mexicana* seed oil on lipase production

The effect of different concentration of *A. mexicana* seed oil range from 1 to 10 % (v/v) on lipase production by mutant *B. pumilus* UVM was studied. Maximum lipase production (58.56±0.03) was observed at 7% (v/v) *A. mexicana* seed oil supplemented basal medium **(Figure 2)**. Further increase in the concentration of *A. mexicana* seed oil there was no significant effect on lipase production by newly isolated mutant *B. pumilus* UVM. Hence, the 7% (v/v) *A. mexicana* seed oil was found as best concentration and used for further study. To date, no attempts have been made to produce lipase from agriculture weed *A. mexicana* seed oil. This is the first report on lipase production from agriculture weed *A. mexicana* seed oil in SmF using newly isolated mutant *B. pumilus* UVM.

Table 3: Effect of UV-C irradiation on selected strain KMAS7

UV-C Irradiation time(min.)	No. of cells/mL after irradiation	Survival percent	Percentage kill
Control (0)	$2.2X10^{7}$	100	0
2	$5.4X10^{6}$	24.54	75.45
4	$4.3X10^{6}$	19.55	80.44
6	$7.2X10^{5}$	3.27	96.72
8	$6.8X10^{4}$	0.31	99.69
10	$6.5X10^{4}$	0.29	99.71

All values (No. of cells/mL after irradiation) are represented as mean ± SD of three replications

Table 4: Screening of extracellular lipase producing mutant strain

Culture Code	Lipase activity (UmL^{-1})
Parent strain - KMAS7	27.12±0.12
UVM1	28.12±0.05
UVM2	28.22±0.02
UVM3	29.15±0.02
UVM4	26.59±0.06
UVM5	34.15±0.04
UVM6	33.45±0.02
UVM7	33.25±0.03
UVM8	31.02±0.01
UVM9	20.33±0.02
UVM10	28.54±0.04
UVM11	21.37±0.05

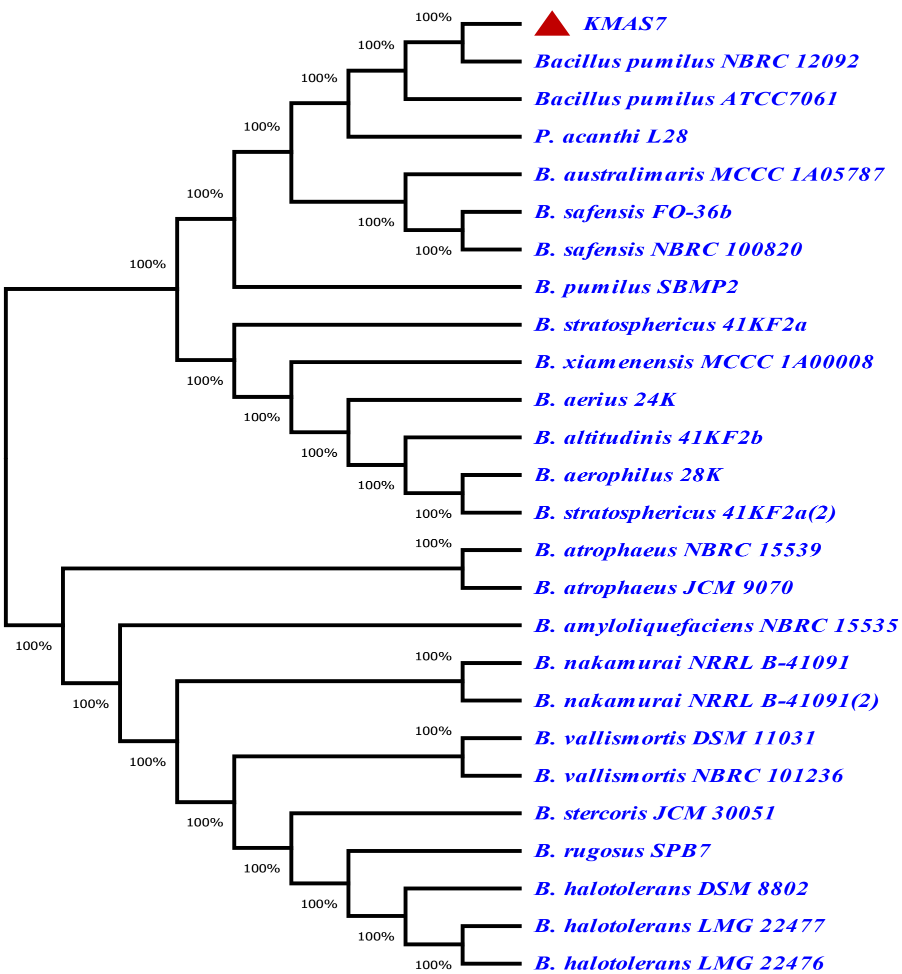

Figure 1: The constructed phylogenetic tree of isolate KMAS7 by Neighbor-Joining method

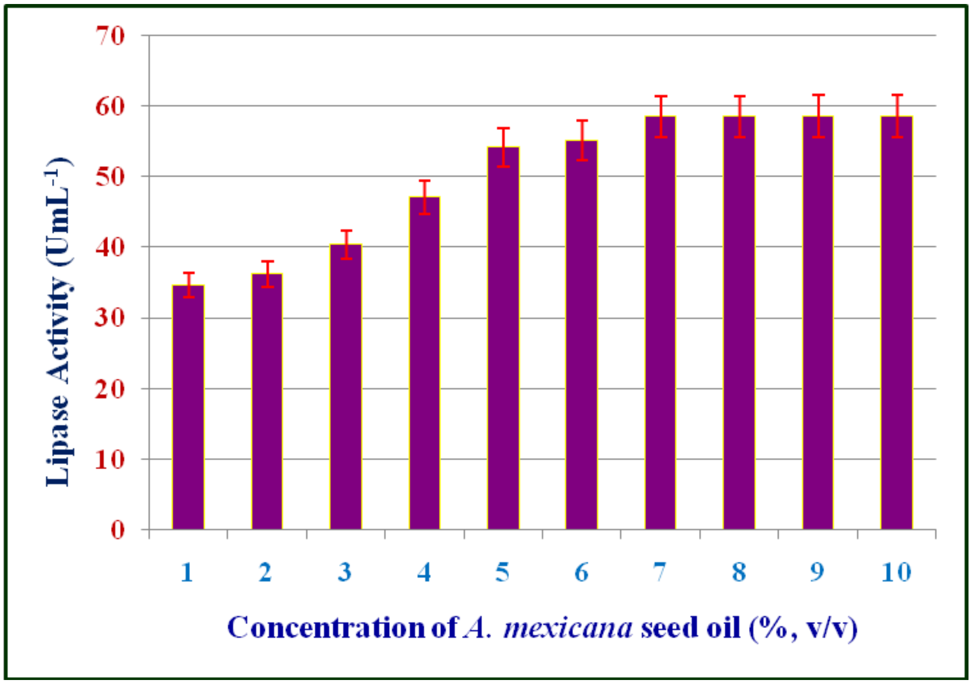

Figure 2: Effect of different concentration of *A. mexicana* seed oil on lipase production by *B. pumilus* UVM

Effect of addition of carbon sources on lipase production

Carbon sources are essential substances to produce energy in microorganisms, especially in bacteria. The ability of an organism to drive a metabolic reaction and develop in the presence of a particular source of carbon depends on the cells typical enzymatic machinery **[35]**. In microbial organisms, carbon catabolite regulation catabolizes carbon to provide carbon and energy optimally for growth **[36, 37]**. In the present study, five different carbon sources such as glucose, fructose, sucrose, maltose, and starch were supplemented into the production media at 0.5 % (w/v) to study their influence on lipase production in submerged fermentation using 7% (v/v) *A. mexicana* seed oil as inducer. As shown in the **Figure 3**, among the five different carbon sources used, the maximum lipase activity 60.25±0.05 UmL^{-1} was obtained in glucose supplemented medium. Whereas other carbon source such as fructose, sucrose, maltose, and starch showed inhibitory effect on lipase production by mutant strain *B. pumilus* UVM. In this study, it was observed that the glucose was utilized by *B. pumilus* UVM for the maximum lipase production in the presence of 7% (v/v) *A. mexicana* seed oil. The newly isolated mutant *B. pumilus* UVM simultaneously utilized two carbon sources (glucose and *A. mexicana* seed oil) for lipase production. At the same time this strain could not utilized fructose, sucrose, maltose, and starch with *A. mexicana* seed oil. This study was accordance with report of Balaji *et al* **[38]**; they found that the glucose and olive oil as best carbon sources for enhanced lipase activity. However, excessive glucose concentrations inhibited enzyme synthesis **[39]**. In the present investigation, the carbon source glucose (0.5%, w/v) supported the growth of *B. pumilus* UVM as well as maximum lipase production. Hence, it was used as best carbon source for further study.

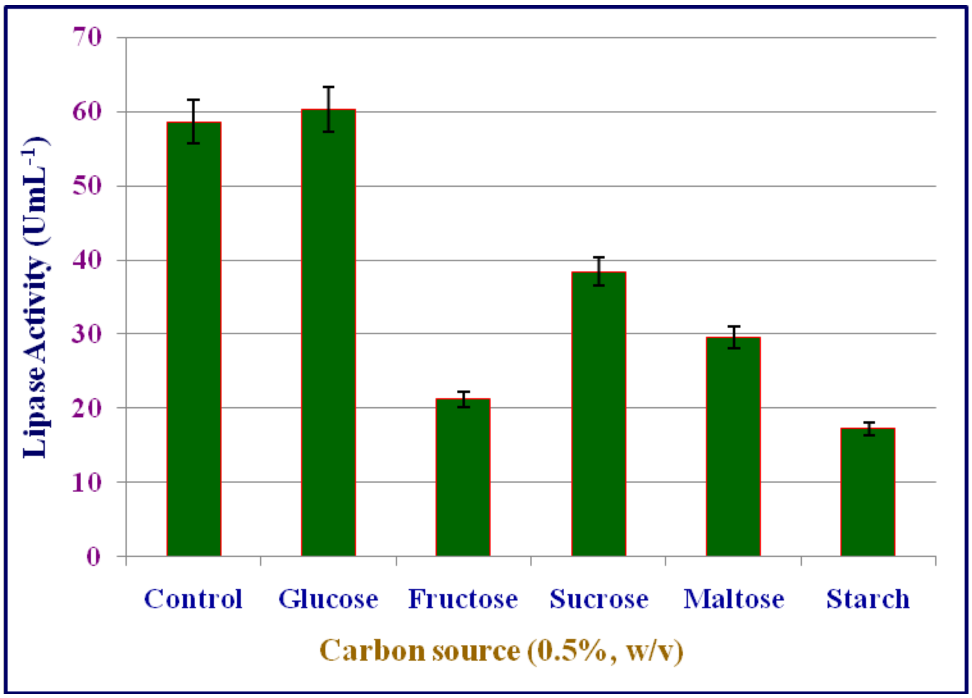

Figure 3: Effect of addition of carbon sources on lipase production by *B. pumilus* UVM

Effect of addition of nitrogen sources on lipase production

Lack of nitrogen source results in decreased protein synthesis and inhibit on of lipase synthesis **[40]**. Both organic and inorganic nitrogen sources have reported to supply nitrogen, cell growth factors and amino acids necessary for enzyme synthesis **[41, 42]**. 0.5%(w/v) glucose and 7% (v/v) *A. mexicana* seed oil contained nitrogen free production media was amended with 1% (w/v) of various nitrogen sources include beef extract, yeast extract, peptone, ammonium chloride and ammonium sulphate and studied their influence on lipase production in submerged fermentation. As shown in the **Figure 4**, maximum lipase production was observed as 63.15±0.02

UmL^{-1} and 62.24±0.04 UmL^{-1} in media containing 1% (w/v) of yeast extract and ammonium sulphate respectively. This study is accordance with studies of Bisht *et al* **[43]** and Mazhar *et al*., **[31]**. The next highest lipase activity was observed in peptone (61.24±0.03 UmL^{-1}) and ammonium chloride (60.11±0.05 UmL^{-1}) supplemented medium. Whereas *B. pumilus* UVM showed less activity in the presence of beef extract (21.23±0.06 UmL^{-1}). Hasan and Hameed **[44]** and Mazhar *et al* **[31]**, showed similar results. Since the cost of yeast extract was higher than that of ammonium sulfate, 1% (w/v) NH_4SO_4 was used as nitrogen source in further study.

Effect of addition of metal ions on lipase production

The effect of various metal ions such as Fe, Cu, Se, Ni and Zn on lipase production from *A. mexicana* seed oil by newly isolated mutant *B. pumilus* UVM was studied by supplementing 10 μML^{-1} of each metal ion individually in the production medium. No metal ion was added while all other experimental procedure was the same for the preparation of control. As shown in **Figure 5**, maximum lipase was obtained in the medium supplemented with 10 μML^{-1} Ni (62.48±0.05 UmL^{-1}) followed by 10 μML^{-1} Zn (61.58±0.04 UmL^{-1}). When compare to control, 2.81-fold increase was observed from these metal ions. While, other metal ions i.e., Fe (60.22±0.02 UmL^{-1}), Cu (59.32±0.05 UmL^{-1}) and Se (57.15±0.03 UmL^{-1}) were supported the lipase production from *A. mexicana* seed oil by newly isolated mutant *B. pumilus* UVM. Nevertheless, the maximum amount of lipase production was obtained from 10 μML^{-1} Ni presented medium. Hence, 10 μML^{-1} Ni was used for further study. This study is in accordance with Akhter *et al* **[37]** and Christianah *et al* **[45]**. However, this studies in contrast with report of Prasad and Manjunath **[46]**. They reported that the metal ion Ni^{+} did not support the lipase production by *Bacillus sp.1* (SAN-L1*), Bacillus sp.* 2 (SAN-L15), *Serratia sp*. (SAN-L21), *Pseudomonas sp*. (SAN-L7) and *Staphylococcus sp*. (SAN-L11) which were isolated from industrial effluents. No clear pattern has been studied in the literature regarding the effect of metal ions on the lipase production from bacterial sources. The effect is different in different metals, and it may even vary with the same metal if the lipase is from a different source.

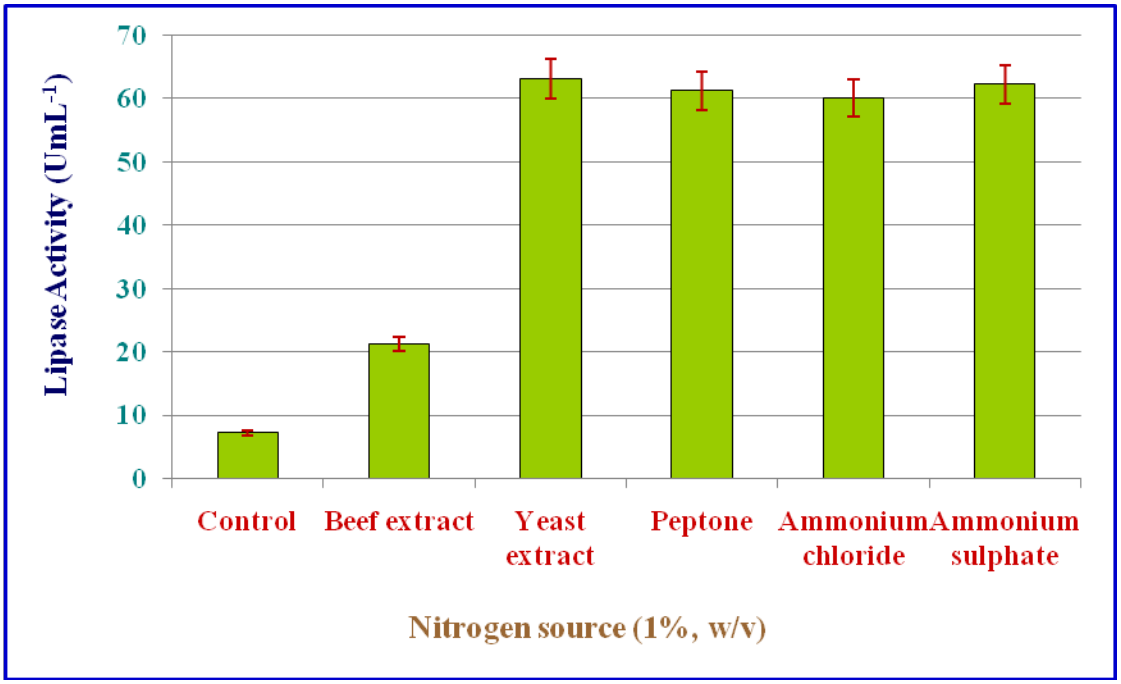

Figure 4: Effect of addition of nitrogen sources on lipase production by *B. pumilus* UVM

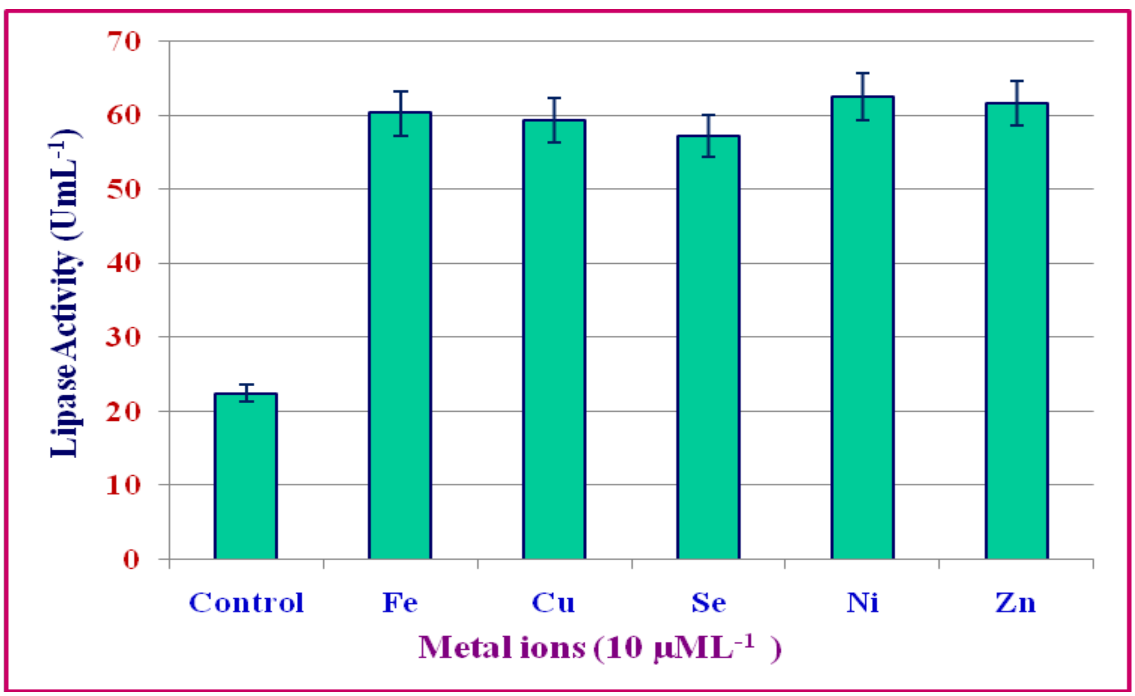

Figure 5: Effect of addition of metal ions on lipase production by *B. pumilus* UVM

CONCLUSION

The bacterial strain KMAS7 was isolated from edible oil contaminated soil samples as a potential lipase producer by utilizing agriculture weed *A. mexicana* seed oil. Based on the morphological and biochemical characteristics it was identified as *B. pumilus*. Subsequently, 16S rRNA of strain KMAS7 was sequenced and submitted to GenBank under the name *B. pumilus* strain KMAS. It was assigned the accession number OR264494. Phylogenetic study revealed and confirmed that the selected isolate KMAS7 was *B. pumilus*. Using UV-C irradiation, the mutant strain UVM5 was created through mutational breeding and given the name *B. pumilus* UVM. This mutant strain produced maximum lipase of 62.48±0.05 UmL^{-1} in basal medium contained 7 % (v/v) *A. mexicana* seed oil as inducer with 0.5% (w/v) glucose, 1%, (w/v) ammonium sulphate and 10 μML^{-1} Ni. The newly isolated mutant *B. pumilus* UVM was able to produce an overall 2.48-fold increase in lipase production from *A. mexicana* seed oil under optimal nutritional conditions. This is the first report on utilization of agriculture weed *A. mexicana* seed oil for lipase production in SmF by newly isolated mutant *B. pumilus* UVM. The findings of this investigation suggest that the agriculture weed *A. mexicana* seed oil is best lipase inducer, which may be utilized to produce lipase on a large scale and at a reasonable cost using bacterial strains.

Conflict of Interest

The authors declare no conflict of interest

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