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Isolation and Identification of Lipase Producing Bacteria From Oil-contaminant Soil

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Abstract
The novel lipase producing isolates from oil contaminated soil were selected. the isolates were collected from different sources . different solid media were used . the lipase activity of crude enzyme were determined. . eight isolates showed high lipase activity . the isolate *pseudomonas aeruginosa* strain showed maximum activity. the 16S rRNA gene sequence analysis were used to identify the eight isolates.
Keywords: Lipase producing bacteria, Isolation, Molecular identification

1.Introduction

Lipases are serine hydrolases that catalyze both hydrolysis and synthesis of long-chain triacylglycerols. Lipases are occupy a place of prominence among biocatalysts owning to their ability to catalyze a wide variety of reactions and are an important group of biotechnologically relevant enzymes and they find massive applications (Mohammed, 2013). lipases are carboxylic ester hydrolases currently attracting an enormous attention due to they are the most versatile and widely used enzyme in biotechnological applications and owing to their unique properties(Cristian,2005). Lipases are produced by microorganisms(bacteria and fungi), plants and animals, However ,microbial lipase especially from bacteria are more useful than their plant and animal origin. since they have great variety of catalytic activities and microorganisms are easy to manipulate genetically and capable of rapid growth on inexpensive media(Sirisha, 2010; Veerapagu *et al* 2013; Mongkolthanaruk and Boonmahome, 2013; Veerapagu *et al*,2014; Jinyong, 2014;). the microbial lipases are commercially most important mainly are secreted into the culture medium by many of microbial species are belong to bacteria, fungi, yeasts and actinomyces(Jeager and Reetz, 1998; Babu and Rap, 2007; Abada, 2008).

Lipases play a vital role in the manufacturing and services sectors for the mankind . Microbial lipases have gained special industrial attention due to their selectivity, stability and broad substrate specificity. Microbial enzymes are also more stable than their corresponding animal and plant enzymes and their production is more convenient and safer(Veerapagu *et al*, 2013).

Generally, bacterial lipases are glycoproteins but some extracellular bacterial lipases are lipoproteins(Abdul-Hammid *et al*,2013; Sagar *et al*,2013).The production of extracellular lipases from bacteria is greatly influenced by medium composition besides physicochemical factors such as temperature, PH and dissolved oxygen . the major factor for the expression of lipase activity has always been reported as the carbon source, since lipase are inducible enzymes. These enzymes are generally produced in the presence of a lipid such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, Tweens, glycerol and bile salts .However, nitrogen sources and essential micronutrients should also be carefully considered for growth and production optimization(Veerapagu *et al*., 2013).Considering the important of lipase enzyme, the present study was aimed to isolation and identification of a novel lipase producing bacteria from different sources of oil-contaminant soil and study the activity of these isolates.

2. Methods and Materials

2.1. Collection of Samples:

For the present study oil-contaminant soil samples were collected from different sources, such as soil exposed to different oils for long periods ,cooking oil, engine oil, garbage site comprising waste from kitchen ,restaurant and fats .the samples were collected from a depth of 5-10 cm using a sterile spatula and stored in sterile glass vials .following collection ,the sample ware immediately transferred to the laboratory for examination and subsequent analysis.(Sagar *et al* 2013)

2.2. Isolation of Lipase Producing Bacteria:

The collected samples ware enriched by periodic sub-culturing of samples in nutrient broth(NB) medium composed from (5gm pepton and 3gm yeast extract)per liter ,pH of medium was adjusted to 7 with 0.1M NaoH.1gm of soil sample was dissolved in 10ml of sterile nutrient broth in 50ml Erlenmeyer flask and agitation at 120 rpm for 30min at 37℃ on a rotary shaker .the sample was serially diluted up to 10^{-6} dilution using 0.8% saline.100µl of each dilution was spread on nutrient agar plate(NA),and incubated at 37℃ for up to 72hr. microbial colonies ,which appeared on nutrient agar plates ware isolated and screened for lipase activity and then subjected to identification (Veerapagu *et al*, 2013;Sagar *et al* 2013).

2.3. Secreening Lipase Producing Bacteria

A sensitive and specific plate assay for detection of lipase producing bacteria makes use of Rhodamine-olive oil-agar(ROA) medium(Ameri *et al*., 2015;Veerapagu *et al*., 2014;Rabbani *et al.,*2013).the growth medium contained(g/L):nutrient broth,8.0;NaCl,4.0;and agar-agar 20.the medium was autoclaved after adjusted to pH 7, and cooled to about 60℃, then ,31.25ml of olive oil and 500 µl % of rhodamine B solution(0.01%w/v distilled water and sterilized by filtration) was added with vigorous stirring .it was the poured into petri plates under aseptic conditions and allowed to solidity (Boonmahome and Mongkoltharuk,2013)the bacterial cultures were inoculated onto these plates. Lipase producing strains ware identified on spread plates after incubation for 48hr. at 37℃. the hydrolysis of substrate causes the formation of orange fluorescent halos around the bacterial colonies or presence of orange fluorescent colonies present on the plates observed under UV irradiation(Aknobi *et al*., 2010; Duza and Mastan, 2014; Sagar *et al* 2013).the colonies with high fluorescence were selected for further studies and quantitative lipase activity assay by titration(PH-stat method) (Rabbani *et al*., 2013).

2.4. Secondary Screening of Lipolytic Bacterial Strains:

The lipolytic bacteria were typically detected were screened through the appearance of white zones by using a selective medium based on Tween80 hydrolysis composed of (g/L) pepton,10; NaCl,5; CaCl.2H2O,0.1;agar-agar,20;Tween80,10ml(v/v)

(Duza and Mastan,2014). the medium autoclaved at 121^{o}C, for 15 min and cooled to 45^{o}C under laminar air flow and added 1%(v/v)Tween80(sterilized by filtration) after autoclave process. These sterile agar plates were punched aseptically with sterile cork borer to obtain 4mm dia. wells in the four halves of the plates with approximate separation distance of 15mm. These agar plates were loaded with suspension of lipase producing bacteria of 0.1ml in each well separately for each isolates and incubated at 37^{o}C in the incubator for 48 hrs. The developed clear zones around the wells were measured (mm) and the data was used for further analysis along with titrimetric assay data to screen the primary isolates for lipase producing bacteria.

2.5. Identification of Lipase Producing Bacteria

The isolate which shows maximal lipase activity on ROA plates assay are considered as positive colonies for lipase enzyme production, these isolates were identified by sequencing of 16s rRNA gene.genomic DNA of the isolates were extracted by using promega Kit(american manufacturing company),the procedure was made according to the genomic DNA purification Kit supplemented by the manufacturing company. Genomic DNA was determined by agarose gel electrophoresis (Sambrook and Russell, 2001).the 16sr RNA gene from the genomic DNA was amplified by PCR using the forward and reverse primers of 16s rRNA(Bioneer, south korea),B 27F(5'-AGAGTTGATCCTGG-'3) and U 1492R(5-'GGTTACCTTGTTACGTT-'3).the PCR was performed in a thermocycler with program comprised intial denaturation at 92 ^{o}C for 2 min. followed by 31 cycles each of 94 ^{o}C for 30 sec.,51.8 ^{o}C for 45 sec.,72 ^{o}C for 1.5 min. and 72 ^{o}C for 5 min. .both strands of PCR product was detected by agarose gel electrophresis,5µl of PCR product was transfered into the wells of agarose gel and in the first well was for
(10 µl)DNA Ladder(1Kb).

The *16SrDNA* products sequencing and its preparation were done according to MACROGEN Co.(Korea), the PCR product volume for each isolate was (20 µl) and then sending to MACROGEN for sequencing . The identity of the sequence obtained was established by comparing with the gene sequences in the database using BLAST software provided by the National Center for Biotechnology Information Service (NCBI) http://www.ncbi.nlm.nih.gov after treatment and recorrection (Kerbauy *et al*., 2011).

3. The Results and Discussion

3.1. Isolation and Screening of Lipase Producing Bacteria

In this study, the soil samples were examined for the presence of lipase positive strains using screening method which is suitable for detection of lipase producers as described in methods. The investigation of lipase activity on Rhodamine-B plates containing olive oil, lipase producer strains were identified by formation of colonies are showed orange fluorescence color when exposed to U.V. light at 350nm after 24hr incubation and formation of orange-colored fluorescent halos around the colonies in long incubation periods(Figure 1)while the colonies that appeared as pink colonies are considered lipase negative strain(figure 1). Eight bacterial isolates from different sources of soil samples were screened as lipase producing bacteria Agar plates containing olive oil and rhodamine B are opaque and pink colored. Lipase producing bacteria forms orange fluorescent halos around their colonies under UV light. this appearance is caused by a complex formation between cationic rhodamine B and uranyl fatty acid ion,the mechanism of this appearance may be related the generation of excited dimmers of rhodamine B which fluoresce at longer wavelengths than the exited monomer(Ameri *et al*.,2015;Boonmahome and Mongkolthanaruk ,2013;Mohammed,2013). Rhodamine-B dye test gave more convenient results than others, Because this test show positive results to only lipase existence and not affected by bacterial metabolite

wastes(Yapasan,2008). Various combinations of substrates like tributyrin and tweens and dyes such as Victoria blue B and night blue can be used in these methods(Adan, 2009) However, These substrates are not suitable to detect true lipases because they are hydrolyzed by esterases, too. The formation of clear zones around colonies against an opaque background on tributyrin agar lacking Victoria blue B indicates lipolytic microbes, but in the presence of Victoria blue B lipolytic colonies are surrounded by dark zones against an opaque , light blue background. The Rhodamine B plate method is not affected by pH changes and does not inhibit the growth of test microorganism or change its physiological properties(Yapasan,2008).

the eight selected isolates were subjected to rapid screening by using Tween80-agar.the results were showed that all the eight isolates exhibited high clear zones(28.6-29.1)mm with Tween80 in the figure (2,3), the clearly apparition of the zone of hydrolysis around colony were considered as important parameters for selection of lipase producer strains. the evaluation of the lipase efficiency based on the clear zones around colonies were showed that all of them could produce lipase.

In direct observation methods, the formation of clear or turbid zones around colonies, or the production of crystals on the agar surface displays the presence of lipolytic activity. Triolein is used as the substrate in lipase detection methods; however, it is difficult to visualize zones of hydrolysis. When Tweens are used as lipase substrates, clear zones are easy to observe((Adan,2009; Zouaoui *et al*, 2012)

3.2. Identification of bacterial strains:

The 16S rRNA gene sequences of the eight bacterial isolates were compared with the database and it showed that strains are members different genes as *Pseudomonas aeruginosa* strain HBUZL-6 (1230bp)with identiyi 96%, *Bacillus pumilus* strain ATCC 7061 (1192bp) with identity99%, *Aeromonas caviae* strain CIFA-KSG1 (943bp)with identity 96%, *Acinetobacter calcoaceticus* strain IHB B 13654 (1306bp) with identity 97%, *Bacillus pumilus* strain YHH-2(1035bp) with identity 99%,*Bacillus pumilus* strain NRC21 (818bp) with identity 98%, *Staphylococcus epidermidis* strain PBR-19(1197bp) with identity 98%, *Bacillus pumilus* strain ToIr-FT (1166 bp) with identity 99%.

The using of the 16Sr RNA gene with primer B24F and U1492R for all bacterial species to prevent losing of any species. Conventional biochemical tests and commercial identification system as well as phenotypic variants are not included in the level of subspecies and often miss identified(Seifert *et al.,* 2003). In contrast, the high-quality of 16SrDNA sequence database provides excellent identification at the species and subspecies levels, furthermore, it can lead to the recognition of novel specis and non-cultured bacteria (Mellmann *et al*., 2006).

The industrial demand for new sources of lipases with different catalytic characteristics was stimulated the isolation and selection of new lipase-producing strains, lipases producing bacteria are distributed in diverse habitats in soils, water and plants in the fields(Alyaa *et al*., 2013), these organisms are occupy different locations as vegetable oil processing factories(Veerapagu *et al.,* 2013), industrial wastes(Sirisha *et al.,* 2010), dairies(Nivedha *et al.,* 2014), soil contaminated with oil decaying food, oilseeds, compost heaps, coal tips, and hot springs (Wang *et al*.,1995; Sharma *et al.,* 2001). The study of Sagar *et al*.(2013) was suggests that waste contaminated sites such as dumped with kitchen wastes, which are usually comprised of numerous lipid leftovers from processes of cooking and non-cooking, can be serve as excellent breeding grounds for the isolation of lipolytic bacteria of industrial significance.

3.2.1. 16S rRNA Sequencing and Analysis Data:

Table (1) showed out the *16SrDNA* gene equences of the eight isolates, the species of bacterial isolates were identified by *16SrDNA* gene sequencing (comparing with identical reference strain of database) .

Table 1. Sequencing of isolates after data manipulation

isolate	Nucleotide sequence	identity	Original strain	Length(bp)
1	TAAACTGCAGTCGAGCGGATGGAGGGAGCTTGCTCCTGGATTCTTCGGCGGACGGGTGAGTAATGCCTATGAATCTGCCTGGT AGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGCGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTA TCGCATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACCATCCGTAACTGGACTGAGAGGATGA TCAGTCACACTGGAACTGACACTCGGTCCAAACTCCTACCGGAGGCAGCAGTGGGGAATATTGGACGATGGGCGAAAGCCTGA TCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCAGATTGGAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTT GCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCATCCGCGGTAATACGAACGGTGCTAGCGTTAATC GGAGTTACTGGGCGTAAAGCGCGCGTAGGTGGGTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCA AAACTACTGAGCTAGAGGACAGTCTAGGGTGGTGAAATTTACTGTGTAGCGCTGAAATGCGTAGATATATGAAGGAACACCAG TAGCAAACGCAACCACCTGGACTGATACTGACACTGAGGTGCAAATCATGAGGAGCAACCAGGATTAGATACCCTGGTAGTCC ACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTAGTCCGCCTGGGGA GTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC GAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCAT GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGG TGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGG CTCCACACGTGCTCCAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATTGTAGTCCGG ATCGCAGTCTGCAATTGGACTGCGGGAAG	96%	*Pseudomonas aeruginosa* HBUZL-6	1230bp
2	ATGCAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAA GACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGC TGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCTGA GAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACG AAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGAGA GTAACTGCTCGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA AGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGA GGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGG AGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT	99%	*Bacillus pumilus* strain ATCC 7061	1192bp

	ACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCA CTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA TTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGT GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCATGAGATGTTGGGTTAAGTCCCGCAACTAGCGCAACCCTTGATCTTAGTT GCCAGCATTTATTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGCAGGAAAGTTGGGGATGACGTCCAATCATCACGCCC CTTATGACCTGGGCTACCAAGTGGTACAATCGAAAACAACAAAGGGTGGAAAGCCTTAAGGGTTACCGAGTCCCGGAAGTTGT TTCCACTTCATAA			
3	TAAGCTAACTACTTCTGGTCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGGCCGGGAACGTATTCACCGCAACATT CTGATTTGCGATTACTAACGATTCCCACTTCCTGGAGTCCAGTTGCAGACTCCAATCCGGACTACGACACGCTTTTTGGGATTCG CTCACTATCGCTAGCTTGCAGCCCTCTGTACGCCGCCATTGGAGCACGTGTGTAGCCCTGGGCGTAAGGGCCATGATGACTTGAC GTCATCCCCACCTTCCTCCGGTTTATCACCCGGCAGTCTCCCTTGAGTTCCCACCATTACGGGCTGGCAACAAAAGACAGGGGTT GCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTCTGATTCCCGAAGGC ACTCCCGCATCTCTGCAGGATTCCAGACATGTCAAGGCCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCAC CGCTGGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGATTTAACGCGTTAGCTCCG GAAGCCACGTCTCAAGGACACAGCCTCCAAATCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC ACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTTCCTCCAGATCTCTACGCATTTCACCGCT ACACCTGGAATTCTACCCCCTCTACAGACTCAGCTGGACAGTTTTAATGCATTCCAGGTTGAGCCGGGGCTTTCCATCTACTTAT CAACCGCTGCGTGCCTTTACGCCAGTAATCCGATAACGCTTGCCCCTCCGTATACCGCGCTGCTGCACGGAGTAGCCGGGCTTC TCTGCAGTACGTCCAGCCACAGATATAACTCTG	96%	*Aeromonas caviae* CIFA-KSG1	943bp
4	AGTCGAGCGGAGTGATGGTGCTTGCACTATCACTTAGCGGCGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGG ACAACATTTCGAAAGGAATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCTTCGGACCTTGCGCTAATAGATGA GCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCAC ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCC ATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTGAAGTTAATACCTTCAGATAG TGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGATT TACTGGGCGTAAAGCGCGCGTAGGCGGCTAATTAAGTCAAATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTCGATACT GGTTAGCTAGAGTGTGGGAGAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGC GAAGGCAGCCATCTGGCCTAACACTGACGCTGAGGTGCGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATG CCGTAAACGATGTCTACTAGCCGTTGGGGCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTA CGGTCGCAAGACTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAA GAACCTTACCTGGCCTTGACATAGTAAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTTACATACAGGTGCTGCATGGC TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTCCTTATTTGCCAGCGAGTAATGTCG GGAACTTTAAGGATACTGCCATTGACAAACTGGAGGAAGGCGGGGACGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTA CCACCGTGCTACAATGGTCGGTACAAAGGGTGGTACCTAACGATAGGAAGCTAATCTCAAAAGCCGATCTTATCCCGGATTGG ATCTGCGACTCTGCCCCCTGAAGTCCGAACCCCCGGAATCCCGGAATTAAGTGCCCGTGGAAAACGTTCCGGGCCTTGCAACCC GCCTTCCCCCGGAGGATTTGTTGCCGAAAGGAGTTCTAACCAAGGACTTCACTGCAAGATTGTTCGATAGGATCAAAAAAAAA GGGGGAAAAGGGGGGGCCCTACCCGAACAAAGAGATTATTGGAATTCCCGGCAGGAACGATGGGGAGAGTAGGGAAGGGAA GTGAACTGAAGAAGGTAGATGTGAAAAGAGGATTGGAGGGGGGGGGGGCCTGGAAGAAAAACGCGGAGGACCCAGAAGGAG CCGGAGAGAGTGGGAGGGAGGTAGGGGGATTAAGGGTAGAAGGGGTGAGAA	97%	*Acinetobacter calcoaceticus* IHB B 13654	1306bp
5	AGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACT GGGATAACTCCGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTC ACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGG GTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAG TCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGAGAGTAA CTGCTCGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCG TTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGT CATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGA ACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCT GGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCG CCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA AGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAG GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGG	99%	*Bacillus pumilus* strain YHH-2	1035bp
6	ACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGGCTGTAAGACTGGGATAACTC CGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGATCACTTACAGA TGGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGC CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA GCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGAGAGTAACTGCTCGCA CCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGA ATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAA CTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGCGAAATGCGTAGAGATGTGGAGGAACACCAGTG GCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGAGGAGCCAACAGGATTAGATACCCTGGTAGTCCA CGCCGAATACGATGAGTCCTAAGTGATAGGGGCTCCCGCTCCGTAGTGATGCAGCTAACGCATTAATCGTCGCTTCACTTCACT CTAAGGAATCACCGGCGACCCCTTACCCCCGTAATTCTGGTTACTTCGACCCCTACGAATACCGTGGCTGG	98%	*Bacillus pumilus* NRC21	818bp
7	ACAGACGAGGAGCTTGCTCCTCTGACGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACC TTCGGGAAACCGGAGCTAATACCGGATAATATATTGAACCGCATGGTTCAATAGTGAAAGACGGTTTTGCTGTCACTTATAGAT GGATCCGCGCCGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCC ACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAG CAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAATGTGTAAGTAACTATGCACGT CTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAA TTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT GGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGC GAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACG CCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTA CGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGA AGAACCTTACCAAATCTTGACATCCTCTGACCCCTCTAGAGATAGAGTTTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCAT GGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACCAGCGCAACCCTTTAACCTTATTTGCCATCATTTAATT TGGGGAATCTAAATTGACTGCCGGTGACAAACCCGAAGAAAGGGGGGGAATAACGTCAAATCATCCAGGCCCCCTATAAATTT GGGGTACAAACGGGGGTACAAATGGAAAATATAAAAGGGGGGCTAAAACCC	98%	*Staphylococcus epidermidis* PBR-19	1197bp
8	CAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCC GGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATG GACCCGCGGCGCATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC AACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGAGAGTAACTGCTCGCACC TTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAAT TATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACT GGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGC GAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACG CCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTA CGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGA AGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATG GTTGTCGTCAGCTCGTGTCATGAGATGTTGGGTTAAATCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCACCATTTATTTGG GCACTCTTAAGGTGACTGCCGGTGACAAACCCGAAGGAAGGTGGGGATGAAGCCCAAATTATCAAGGCCCCTTATTAACCTGG GGTTAACACACTGGTTAAAATGAGAAGAAAGAAAATGCGTTCCAAGAACCCC	99%.	*Bacillus pumilus* TolR-FT	1166 bp

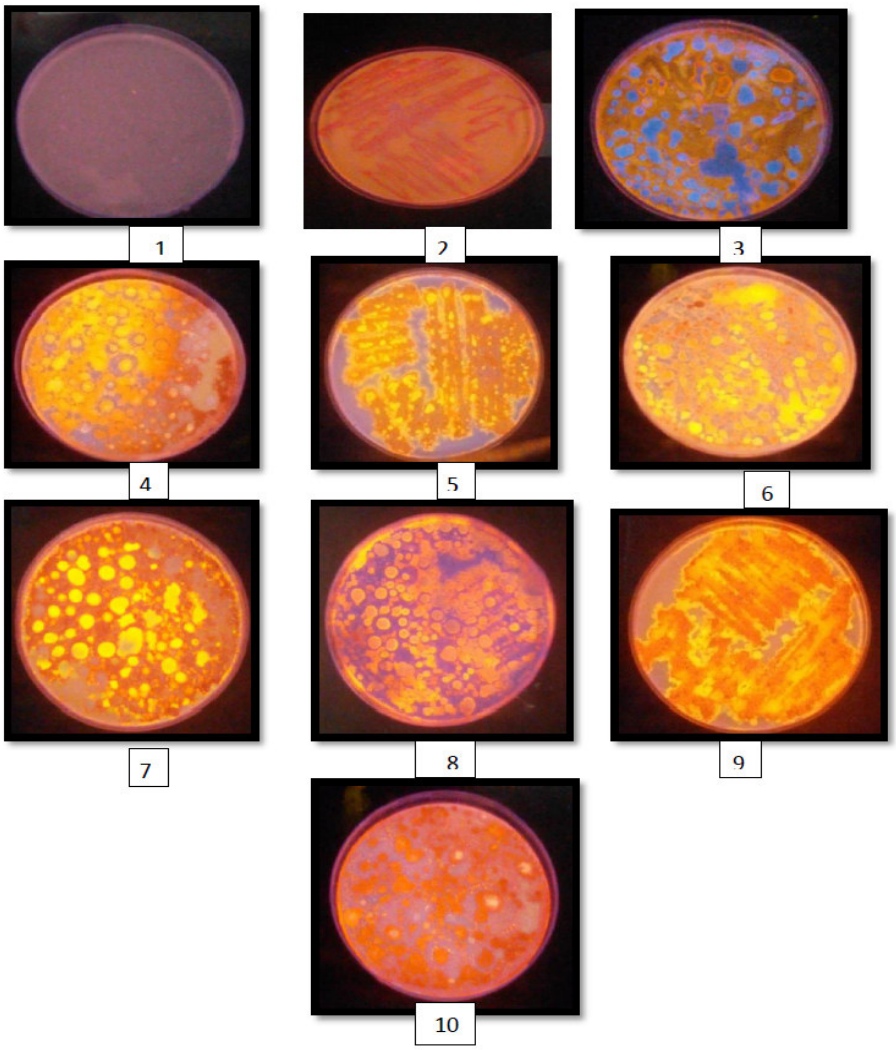

Figure 1. Cultivation of The Lipase Producing Bacteria on Rhodamine B plates, Showed Under UV Light at 350nm.1-blank control. 2- negative control. 3-pseudomonas aerignosa. 4-Bacillus pumelus stain ATCC 7061. 5- Aeromonas caviae.6- Acinetobacter calcoaceticus. 7- Bacillus pumilus strain YHH-2. 8- Bacillus pumilus strain NRC21 .9- Staphylococcus epidermidis.10- Bacillus pumilus strain ToIr-FT

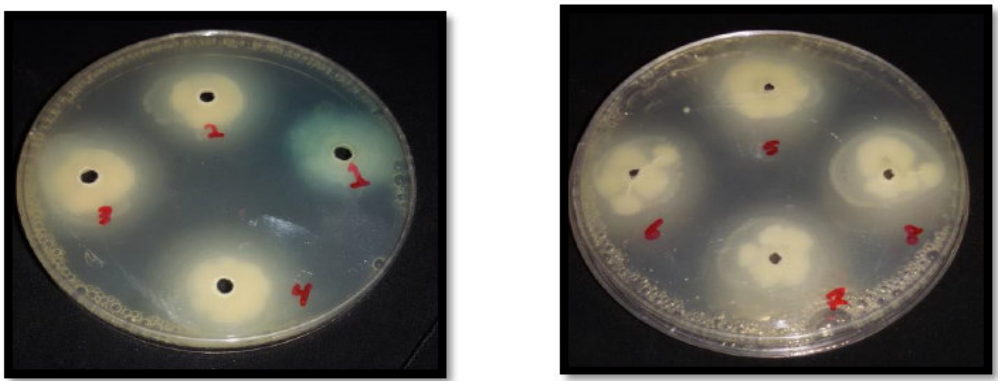

Figure 2. Cultivation of The Lipase Producing Bacteria on Tween 80 1-*pseudomonas aerignosa.*2-*Bacillus pumelus* ATCC 7061.3- *Aeromonas caviae*. 4-*Acinetobacter calcoaceticus.*5- *Bacillus pumilus* strain YHH-2. 6- *Bacillus pumilus* strain NRC21.7- *Staphylococcus epidermidis.* 8- *Bacillus pumilus* strain ToIr-FT

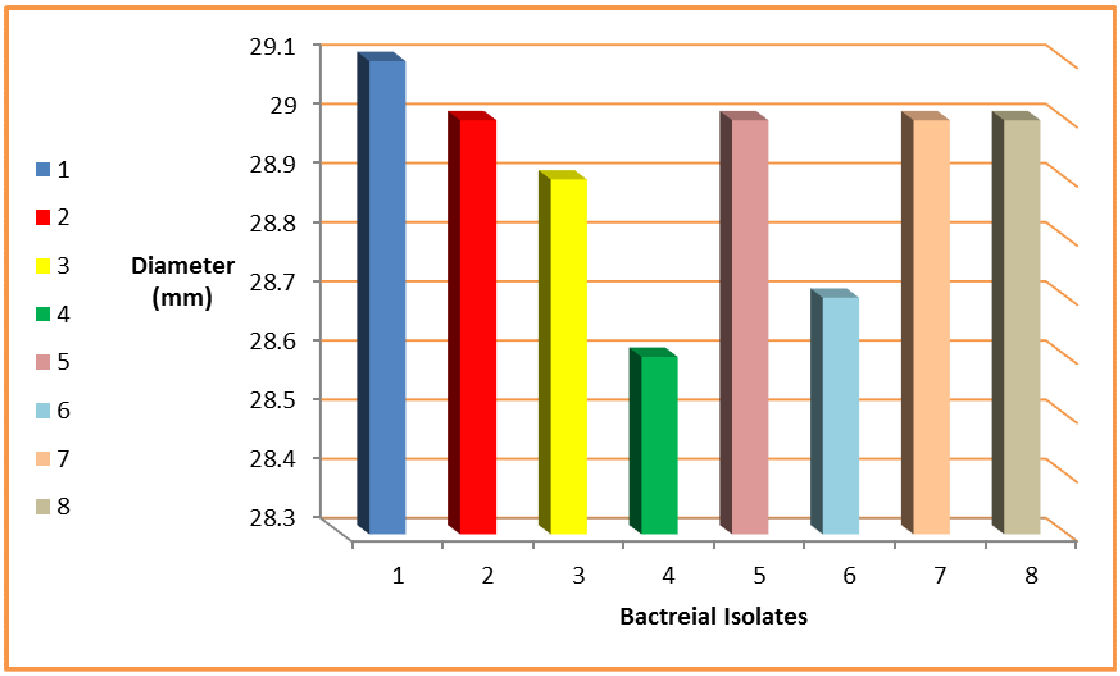

Figure 3. The Diameter Ratio of Clear (White) Zone and Colony in(mm) of the Lipase Producing Bacteria on Tween80: 1-*Pseudomonas aerugnosa.* 2-*Bacillus pumelus* ATCC 7061 .3- *Aeromonas caviae*. 4-*Acinetobacter calcoaceticus* .5- *Bacillus pumilus* strain YHH-2. 6- *Bacillus pumilus* strain NRC21.7- *Staphylococcus epidermidis.* 8- *Bacillus pumilus* strain ToIr-FT

Conclusions

The soil is a reservoir of a huge and diverse microbial population, which is considered a rich source of many types of microbial strains which can afford a particular group of microbial strains necessary for the degradation of different contaminants thrown into the soil. Hence the soil samples may be used to isolate the novel strains that can be used as a part of the microbial collection for the production of lipase at research labs and industries.

All the bacterial isolates as *pseudomonas aerignosa, Bacillus pumelus* stain ATCC 7061, *Aeromonas caviae* , *Acinetobacter calcoaceticus*, *Bacillus pumilus* strain YHH-2, *Bacillus pumilus* strain NRC21, *Staphylococcus epidermidis, Bacillus pumilus* strain ToIr-FT were isolated during the survey from oil-contaminant soil. and all the isolates were exhibited the lipase producing activity. the isolates *pseudomonas aeruginosa* showed the highest lipase production activity . the use of Rhodamine B-plate agars as a good media to study lipase producing activity. So the using of 16SrDNA sequence database provides excellent identification at the species and subspecies levels and it can lead to the recognition of novel species of bacteria.

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