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A STUDY ON LIPID DEGRADING BACTERIA (ISOLATION, IDENTIFICATION, PRODUCTION AND CHARACTERIZATION)

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ABSTRACT: This piece of work starts by describing the function structure and metabolism of lipids and also shows the importance of it, as start lipids are considered a heterogeneous group of molecules that have the common property of hydrophobicity. When we talk about lipids we may see a wide range in structure from simple to complex hydrocarbon molecules, after that; go further to evaluate the production of lipolytic complexes that can be produced by various types of microorganisms isolated from different types of contaminated soils from many sources such as wastes surrounding factories, slaughters and oily soils at the mechanic shops in order to select the best lipolytic microorganism which can survive in these soil conditions, to be used in biotechnological processes. We tested 14 types of bacterial isolates taken from the previous places. Lipase production had been assayed in Enriched media, Selective media and Differential media with agar-based substrates. Then samples were incubated at 30 ^{o}C and 25 ^{o}C for 24 hrs. Growing showed 5 lipase producers.The optimization process was followed by culturing these bacteria in different conditions" temperature, pH" to select the one which have the highest yield.Isolated strains were then going to the DNA gel electrophoresis process in order to confirm the presence of DNA, then the DNA is taken for a PCR to multiple the strand of DNA, after the DNA send for sequencing identified according to the 16S RNA and It revealed that these strains belong closely to *bacillus cerus, pantonea sp., pseudomonas statceri andbacillus meaterium* which show the highest lipolytic activity.

Key words: Lipase, Lipolytic microorganisms, contaminated soils, screening, substrates.

INTRODUCTION

Lipase is a glycerol ester hydrolase that acts on acylglycerol to release fatty acids and glycerol. Lipase can hydrolyze long- chain water-insoluble triglycerides into diglycerides, monoglycerides, glycerol, andfatty acids. Lipase is a widely used abundant enzyme (**Javed *et al.*, 2017**).

Spread in animals, plants and microorganisms. The capability of lipase to make highly specialized chemical transformations makes it increasinglypopular in various fields of industries such as food, detergents, cosmetics, and pharmaceutical industries.

High varieties of microorganisms produce lipase. Most of the commercially usefullipases are of microbial origin. Lipase- producing microorganisms contain industrial waste, vegetable oil processing plants, dairy products, oil-contaminated soil we can see that lipase-producingmicroorganisms are found in a variety of environments, including rotten foods **(Devaraj and Rajalakshmi, 2019)**.

Microbial lipases have received particular industrial attention due to many properties such as selectivity, stability, and wide substrate specificity. The microbial enzymeis more stable than the corresponding plant and animal enzyme, making it more convenient and safer to

manufacture (**Verma *et al.*, 2012).**

Lipolytic microorganisms are recognized to be potential producers of extracellular lipases, including bacteria, yeasts and fungi. Extracellular lipase has been isolatedfrom a wide variety of bacterial species, including Bacillus and Pseudomonas. Particular attention has been paid to thespecific enzyme class of the species Bacillus and Pseudomonas, which was first studied and used in biotechnological production, because it is involved in bacterial pathogenesis (**Hombalimath *et al.*, 2012)**.

A simple and consistent method for noticing lipase activity of microorganisms is described in this method by using a Tween 80 which plays an important role in being a surfactant in solid medium to identify lipolytic activity. The formation of clear zones around the appeared colony indicates lipase production byorganisms (**Abd-Elhakeem *et al.*, 2013)**.

Screening of lipase producers on agar platesis also often complete using tributyrin as a substrate, and a clear zone surrounding thecolony indicates lipase production. A screening system using a chromogenic substrate is also defined. Plates of modifiedRhodamine B agar were used to screen for lipase activity of a large number of microorganisms (**Singh *et al.*, 2017)**.

In general, high productivity isaccomplished by optimizing the medium that makes up the medium is usually a time-consuming procedure. By-products can be used as substrates for lipase production to deliver high quality, low cost substrates and decrease the final cost of the enzyme. Considering the importance of the lipase enzyme, Lipase, which belongs to the genus Bacillus and Pseudomonas were characterized and optimized in this study.

MATERIALS AND METHODS

Collection of samples

Metal spatula was used to dip the selected soil from different sources such as contaminated oily soil surrounding slaughters, factories, shops that have oil waste product under 15 cm depth and 40g of the soil were collected in a well zipped plastic bag and saved in room temperature conditions (**Jharna *et al.*, 2012)**.

Preparing the sample

By taking 2 spatulas from each bag and putting it in distilled water 100 ml and then making a serial of dilutions then putting them inside the shaker for mixing all the ingredients together after that the sample was ready for the next step (**Lehninger, 1975)**.

a. Serial dilution

Serial dilutions were prepared from 1 ml of the aqueous solutions of each sample. For each sample 1 ml of the aqueous mixture was added to a test tube containing distilled water by using a sterile micropipette and labeled as 10^{-1}. 1 ml from this solution was shifted to another test tube having distilled water labeled as 10^{-2}. Correspondingly 1 ml was shifted to another test tube having distilled water and was labeled as 10^{-3}. Taking 1 ml from the prepared flasks and putting it inside the ready-made test tube containing broth, repeating the steps for each sample, then samples were left inside the incubator for 24 hrs. (**Lehninger, 1975).**

Screening the sample

1- Screening on Enriched media

It's a type of media permit the fastidious organism to grow because of the presence of essential

nutrient additives such as blood, serum, hemin, etc. An example of this category is the blood agar, Brain heart infusion agar, nutrient agar and chocolate agar.

2- Nutrient Agar Streaking

Nutrient agar was prepared by suspending 28g of powder in distilled water and by usinga magnetic heater and stirrer the media allow to mix properly and to boil till complete dissolving.

3- Nutrient agar plates

Take a swap from the bacterial growth present in the test tubes and streaks it on the agar plates. Using the spread plate technique which is considered a very viable method for counting the bacteria present in the liquid broth sample,

perfect spread results in a visible isolated colony that is well distributed on the agar plate by using L- shape sterilized (**Aji *et al.*, 2014)**.

4- Brain heart infusion agar

Taking a swap from the bacterial growth present in the test tubes and streaks it on theagar plates. Using spread plate technique which considered very viable method for counting the bacteria present in the liquid broth sample, perfect spread results a visible isolated colony that are well distributed on the agar plate by using L- shape sterilized **Aji *et al.* (2014)**.

b. Screening on substrate-based media

Selected strains are then streaked on the substrate-based agar and we performed several tests such as starch hydrolysis test, phenol red plates (1% tributyrine) test, gelatin hydrolysis test and streaking on olive oil agar, other substrates were used as a carbon and nitrogen source such as: feather and straw **(Chester, 2020)**.

c. Biochemical tests

The biochemical tests were done for further identification of the strains. They are done for differentiation between Gram negative rods and cocci and Gram-positive rods and cocci. biochemical tests used were urease test, citrate test and TSI (triple sugar iron) **(Chester, 2020)**.

d. Secondary screening

According to the preliminary screening, 5 bacterial isolates (named as: S1-5) were subjected to secondary screening using Tween 20 agar plates (containing: peptone (667 g L−1), NaCl (5 g L−1), CaCl2.2H2O (0.1 g L−1), agar–agar (20 g L−1), and 10 mL of (156 g L−1) Tween20). A Loop full of these pure bacterial isolates was separately inoculated on sterilized Tween20 agar plates and raised at 37 ºC for 24 h. After incubation, the colonies were detected and distinguished from the colonies' zone of clearance **(Elhussiny *et al.*, 2020)**.

Preparing the isolates for the DNA Extraction

Isolates are then incubated for 14-16 hrs. at 37 ºC, then picked up a single colony from the agar plate then we inoculate it in 5 ml liquid and incubate it overnight at 37 ºC (**Tripathia *et al.*, 2013)**.

DNA Extraction, gel electrophoresis and PCR test

Isolates that show optimum results are then taken to the next step which is DNA extraction, by following the protocol F, DNA is extracted from different samples and their quality was then measured by spectrophotometer, a fragment of the 16S run gene were then amplified by PCR and purified forward and reverse DNA sequencing reaction was then carried out with primer 1492R which is considered as a universal primer. The sequence obtained was aligned using data software and a phylogenetic tree was constructed (**Tripathia *et al.*, 2013)**.

RESULTS AND DICUSSION

The growth of microorganisms

Isolated colonies showed a significant growth in Nutrient agar and LB broth when we worked inside the airflow laminar and then we put the samples inside the incubator at37 ºC for 24 hrs.

Screening on different selective media and its characterization

Isolated colonies showed high degraded ability on the selective media after performing some tests such as: Starch Hydrolysis tests, phenol red plates (1"% tributyrin), gelatin hydrolysis test, and CBC and LBG test (**Tripathia *et al.*, 2013)**.

Gel electrophoresis bands

The upcoming figure showed theappearance of bands that represent the DNA in the 5 selected isolates. After separation, the resulting DNA fragments are visible as clearly defined bands. The DNA standard or ladder areseparated to a degree that allows for the useful determination of the sizes of samplebands (**Tripathia *et al.*, 2013).**

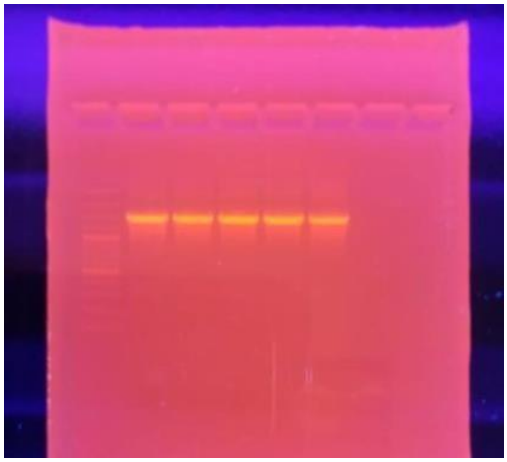

Figure 1. DNA fragments "Agarose Gel electrophoresis"

Identification of the selected isolates

All the products sequencing and its preparation were done according to Macrogen co (Korea), the PCR product volume for each isolate was (20 µl) and then sent to MACROGEN for Sequencing. The identity of the sequenceobtained was established by relating it with the gene sequences in the database using blast software provided by the National Center for Biotechnology Information Service (NCBI) (**Ansell *et al.*, 1973)**.

Analysis data and 16S rDNA Sequencing

It is showed out in Table 2 that the 16S rDNA genesequences of five isolates, the species of the bacterial isolates were then identified and taken from their original strain (**Verma *et al.*, 2012).**

Table 1. Sequencing of isolates after data manipulation

Isolate	Identity	Original strain	length
GP1	96.76%	Bacillusanthracis	1242
GP2	97.25%	Bacillus cereus	1558
GP3	96.93%	Pantoea sp.	1245
GP4	96.89%	Pseudomonasstutzeri	1316
GP5	95.22%	Bacillus megaterium	1571

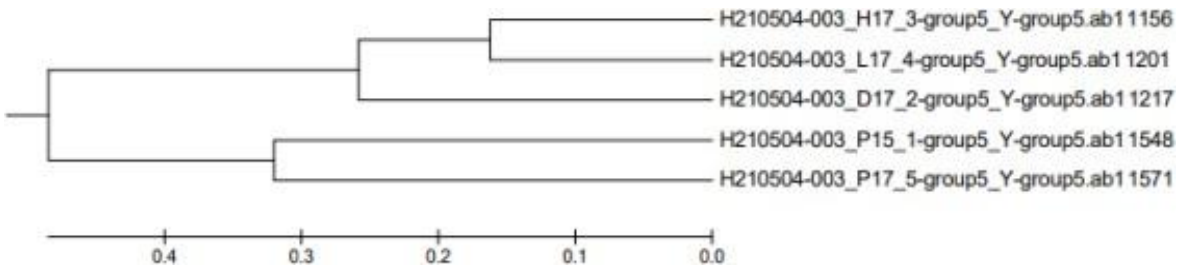

Figure 2. Phylogenetic tree for microorganisms

Lipase Primer

Finally, a specific primer of lipase enzyme has been designed in 2 directions forward and reverses and sent for production (**Bornemann *et al.*, 1989)**.

Lip F2
GGGGATCCGCACCACCACCCTATTCCGCGCTTGGC
Lip R1
GGGGATCCTCAGACTGCTTTTTCCTGGACATCAA

Characterization of lipase enzyme

Several tests occurred to see the effect of different pH and temperature and also the effect ofdifferent substrate concentrations.According to Thomas et al., lipase production varies with species for the parameters like optimum temperature, optimum pH and enzyme specificity (**Brune and Gotz, 1992)**.

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RESEARCH ARTICLE

A Study on Lipid Degrading Bacteria (Isolation, Identification, Production and Characterization)

Authors' contributions

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