St. Joseph's Journal of Humanities and Science
ISSN: 2347 - 5331
http://sjctnc.edu.in/6107-2/

ISOLATION AND CHARACTERIZATION OF LIPASE PRODUCING MICROORGANISMS FROM AGRICUTURAL SOIL AND DIFFERENT WASTES

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Abstract

Lipases enzyme catalyzes the hydrolysis of lipid. Among the various lipases .bacterial lipases are the most significant when compared with animal and fungal lipases. The aim of the present study was to isolate the lipase producing *Bacillus* & *Pseudomonas* strain from various agricultural and soil samples and to study the enzyme activity of lipase by optimization parameters by using various lipid source, different pH and determining the stability of lipase enzyme by immobilization technique. The isolates were selected based on cellular morphology, growth conditions and biochemical tests. The different Bacillus species were screened by using Hicrome Bacillus for Pseudomonas cetrimide agar is used. The results obtained in the present study revealed that lipase producers shows promising and good source of lipase.

Keywords: Lipase, Hicrome Bacillus agar, Bacillus sps, ROA plate assay.

INTRODUCTION

Microorganisms excrete a wide variety of lipolytic enzymes, which are also found in mammalian systems. They are molecules of small size, compact, spherical structures that catalyzes the ester bonds in lipids. commercially they are very important and isolated from various living sources such as plants, animals, bacteria and fungi.

Lipases from microbial sources are preferred over the enzymes from plant or animal sources since they possess all most the characteristics desired for their biotechnological applications, Among bacteria *Bacillus* and *Pseudomonas* species are specific producers of lipases. Lipases have wide application in waste management, recycyling technology, and it is also employed in detergents, foods, beverages, health foods and cosmetics. Microbial lipases have produced by yeasts, fungi and bacteria as extracellular, intracellular, cell bound enzyme, these microbes found in diverse habitat especially in oil processing industries. Lipases are serine hydrolases which has uncommon potential of acting at the lipid-water interface, Different genera of bacteria are known to produce lipase have been well exploited for lipase production (Tembhukar *et al.,* 2012).

MATERIALS AND METHODS

Isolation and identification: Different samples were collected randomly from different sites such as garden

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soil, agricultural soil, diesel soil and different wastes such as dairy waste, canteen waste detergent water, oil effluent, oily water, for this study a total of 19 samples were used and processed in the laboratory by using basic microbiological techniques. The lipid producing bacteria was isolated by using Tributyrin agar and then further it was screened and its phenotypic character was studied by using Hicrome Bacillus agar for Identifying *Bacillus* species and Cetrimide agar for *Pseudomonas* species. The isolates was identified based on cellular morphology, Gram staining, endospore staining and biochemical tests.

ROA PLATE ASSAY

(Rhodamine – Olive oil agar)

A sensitive and specific plate assay for detection of lipase producing bacteria makes use of Rhodamine olive oil agar medium. The growth medium contained(g/l) nutrient broth, Nacl, agar agar. The medium was adjusted to pH 7.0, autoclaved and cooled to about 60°C, then olive oil and rhodamine B solution was added with vigorous stirring. It was then poured into petriplates under aseptic conditions and allowed to solidify. The bacterial culture was inoculated into the medium, lipase producing strains were identified on spread plate method after incubation for 48hrs at 37°C.

OPTIMIZATION PARAMETERS FOR LIPASE ACTIVITY

Optimization was done by using different Lipase Producing Isolates.

Effect of pH on Lipase Production

The isolated culture such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus*, and *Bacillus subtilis* inoculated in tributyrin broth at different pH ranges from pH 4 to 10 and incubated at 37°C for 48 hours, the lipase activity was observed in UV spectrophotometer at 560nm.

Effect of Lipid Source on Lipase Production

The isolated cultures such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus*, and *Bacillus subtilis* inoculated in Tributyrin broth supplemented with various lipid sources such as olive oil, palm oil,gingely oil& castor oil and incubated at 37°C for 48 hours, the lipase activity was observed in UV spectrophotometer at 560nm.

Stability of Lipase Enzyme by Immobilization

The stability of lipase enzyme was studied by immobilization technique by using sodium alginate and calcium chloride solution with the preparation of crude enzymes and immobilized beads were obtained.

Hydrolysis of Starch

The isolated cultures such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus*, and *Bacillus subtilis* inoculated in starch agar plates and incubated at 37°C for 24 hrs.

Hydrolysis of Casein

The isolated cultures such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus*, and *Bacillus subtilis* inoculated in skim milk agar plates and incubated at 37°C for 24 hrs.

Lecthinase Activity

The isolated cultures such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus*, and *Bacillus subtilis* inoculated in egg yolk media and incubated at37°C for 24hrs.

RESULTS AND DISCUSSION

Morphological and Physiological Characteristics

Morphological and physiological characteristics of bacterial isolates of lipase producers were investigated according to the methods described in Bergey's Manual of Determinative Bacteriology. (Table.1) It was identified as a member of *Bacillus* species. The colonial appearance of this *Bacillus* species in Hicrome Bacillus Agar (Plate:1) possess different colonies such as *Bacillus pumilus* were produces light green to green color colonies, *Bacillus subtilis* shows yellowish green to green colonies, *Bacillus megaterium* shows yellow mucoid colonies, *Bacillus cereus* produces light blue, large flat with blue centred colonies. Among these

lipase producers another important Gram negative bacteria such as *Pseudomonas aeruginosa* was frequently isolated during the study. It showed bluish color colonies on cetrimide Agar.

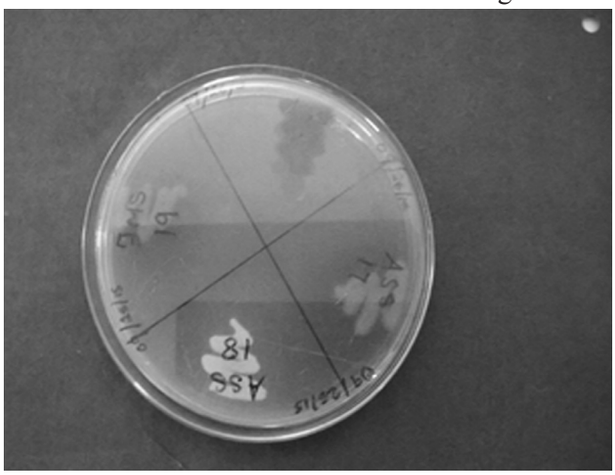


Plate.1: Growth of different Bacillus sps in Hicrome Bacillus Agar

Table.1 Biochemical Characterization

Biochemical Characteristics	B.pumilus	B.subtilis	B.cereus	B.megaterium	Pseudomonas aeruginosa
Cellular morphology& Gram staining	Gram positive rods	Gram positive rods	Gram positive rods	Gram positive rods	Gram negative rods
Spore staining	Spore former	Spore former	Spore former	Spore former	Non Spore former
Activity of Catalase	Positive	positive	positive	Positive	Positive
Activity of oxidase	Positive	positive	positive	Positive	Positive
Indole reaction	Negative	negative	negative	Negative	Negative
Methyl red reaction	Negative	negative	negative	Negative	negative
Voges proskauer reaction	Positive	positive	positive	Negative	Positive
Citrate utilization	Positive	positive	positive	Positive	Positive
Starch hydrolysis	Positive	positive	positive	Positive	Positive
Casein hydrolysis	Positive	positive	positive	Positive	Positive
Lecthinase activity	Positive	positive	positive	Positive	Positive
Urease activity	Negative	negative	negative	Negative	Positive
TSI	k/A	k/A	k/k	k/k	K/k
LIA	k/k	K/K	K/K	k/k	k/k
Mannitol motility test	Nonmotile	nonmotile	nonmotile	nonmotile	motile

ROA PLATE ASSAY

(Rhodamine – Olive oil agar)

Olive oil is the potential lipid source present in the medium, Rhodamine dye is a flourchrome dye which reacts with UV light produces flouresence .The hydrolysis of substrate by lipase producers causes the formation of orange flourscent halos around bacterial colonies visible upon UV irradiation.(Davender kumar *et al.,* 2012) *Bacillus cereus, B.pumilus.B.subtilis,B. megaterium & Pseudomonas aeruginosa* showed lipase activity in this method.

Optimization Parameters of Enzyme Production

Effect of pH: (Mukesh kumar *et al.,* 2012)The following *Bacillus* species such as *B. subtilis, B.cereus, ,B.pumilus* and *Pseudomonas aeruginosa* was allowed to grow in Tributyrin broth of different pH ranging from 4 to 10 . Maximum enzyme activity was observed in pH 7 by *B.cereus* , pH 10 *Bacillus pumilus*,pH 10 by *Bacillus subtilis* and pH 8 by *Pseudomonas aeruginosa*. (Table: 2, 3 , 4 ,5) & (Graph: 1, 2, 3, 4)

Table.2 Effect of pH on Lipase Production by *Pseudomonas aeruginosa*

Sl.No	Tributyrin Broth at different pH	OD at 560nm
1	4	0.4358
2	5	0.5303
3	6	0.6303
4	7	0.977
5	8	1.9186
6	9	0.355
7	10	0.255

Graph.1 Effect of pH on Lipase Production by *Pseudomonas aeruginosa*

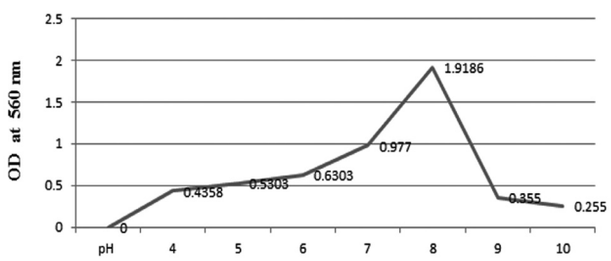


Table.3 Effect of pH on Lipase Production by *Bacillus Cereus*

Sl.No	Tributyrin broth at different pH	OD at 560nm
1	4	1.4587
2	5	0.8485
3	6	2.6480
4	7	3.2213
5	8	2.9186
6	9	2.3770
7	10	1.0536

Graph.2 Effect of pH on lipase production by *Bacillus cereus*

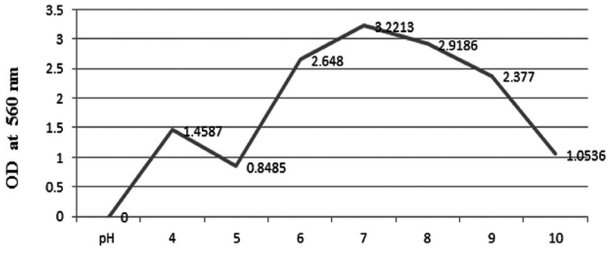


Table.4 Effect of pH on lipase production by *Bacillus pumilus*

Sl.No	Tributyrin broth at different pH	OD at 560nm
1	4	0.8718
2	5	0.3253
3	6	0.2377
4	7	0.1458
5	8	0.0623
6	9	0.2711
7	10	2.5784

Graph.3 Effect of pH on Lipase Production by *Bacillus pumilus*

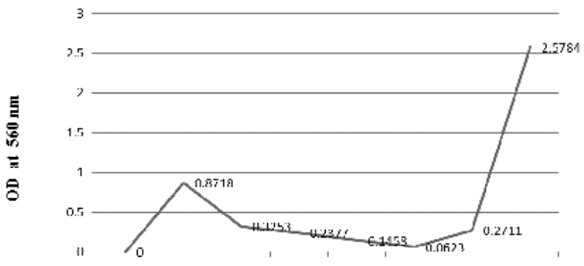
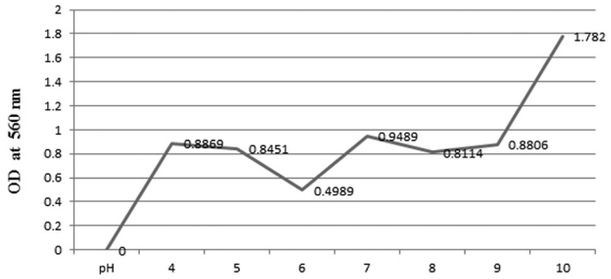


Table.5 Effect of pH on Lipase Production by *Bacillus subtilis*

Sl.No	Tributyrin broth at different pH	OD at 560nm
1	4	0.8869
2	5	0.8451
3	6	0.4989
4	7	0.9489
5	8	0.8114
6	9	0.8806
7	10	1.7820

Graph.4 Effect of pH on Lipase Production by *Bacillus subtilis*



Effect of lipid source: (Patcha Boonmahome *et al.,* 2013**)** various lipid sources such as palm oil, gingely oil, olive oil, coconut oil, castor oil, groundnut oil were added in growth media. Results obtained were showed that *B.subtilis* shows high enzyme activity in castor oil*, B.pumilus* in palm oil, *B.cereus* in gingely oil & coconut oil, and *Pseudomonas aeruginosa* in gingley oil & castor oil. (Table: 6, 7 , 8, 9) & (Graph:5, 6, 7, 8)

Table.6 Effect of lipid source on Lipase Production by *Pseudomonas aeruginosa*

Sl.No	Tributyrin broth with different lipid source	OD at 560nm
1	Palm oil	0.4719
2	Gingely oil	2.2384
3	Olive oil	1.3889
4	Coconut oil	0.8650
5	Castor oil	2.2281
6	Groundnut oil	0.9312

Graph 5. Effect of Lipid Source on Lipase Production by *Pseudomonas aeruginosa*

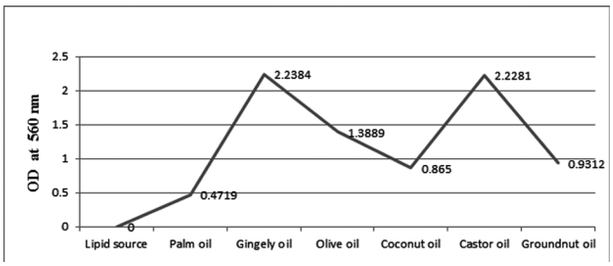


Table. 7 Effect of Lipid Source on Lipase Production by Bacillus cereus

Sl.No	Tributyrin broth with different lipid source	OD at 560nm
1	Palm oil	0.2341
2	Gingely oil	0.8452
3	Olive oil	0.0435
4	Coconut oil	0.9128
5	Castor oil	0.5616
6	Groundnut oil	0.5941

Graph 6. Effect of Lipid Source on Lipase Production by *Bacillus cereus*

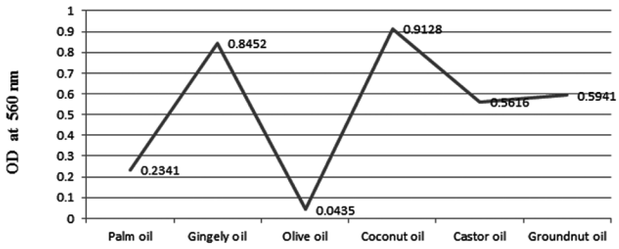


Table. 8 Effect of lipid source on lipase production by Bacillus subtilis

Sl.No	Tributyrin Broth with different Lipid Source	OD at 560nm
1	Palm oil	0.4664
2	Gingely oil	0.5010
3	Olive oil	0.1725
4	Coconut oil	0.3885
5	Castor oil	0.6253
6	Groundnut oil	0.5380

Graph.7 Effect of Lipid Source on Lipase

Production by *Bacillus subtilis*

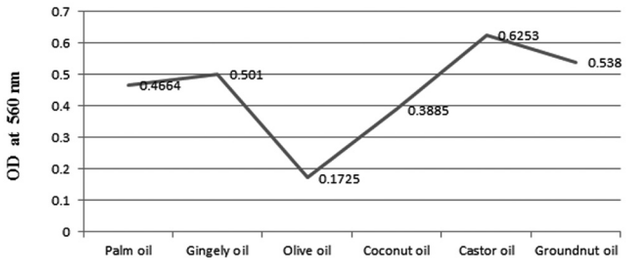
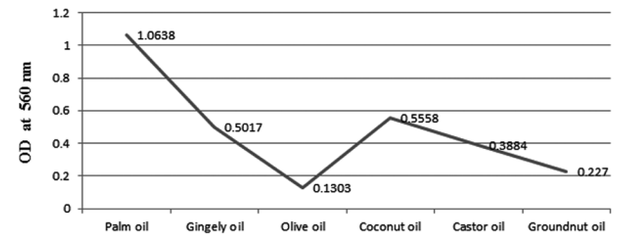


Table.9 Effect of Lipid Source on Lipase Production by *Bacillus pumilus*

Sl.No	Tributyrin broth with different lipid source	OD at 560nm
1	Palm oil	1.0638
2	Gingely oil	0.5017
3	Olive oil	0.1303
4	Coconut oil	0.5558
5	Castor oil	0.3844
6	Groundnut oil	0.2270

Graph.8 Effect of Lipid Source on Lipase Production by *Bacillus pumilus*



Hydrolysis of Starch

The isolated cultures such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus*, and *Bacillus subtilis* inoculated in starch agar plates and incubated at 37°C for 24 hrs. After incubation zone of clearance was observed by using Grams iodine solution, the hydrolysed area forms clear zone and the unhydrolyzed area appears dark brown color.

Hydrolysis of Casein

The isolated cultures such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus*, and *Bacillus subtilis* inoculated in skim milk agar plates and incubated at 37°C for 24 hrs. After incubation zone of clearance was observed indicates the organism has hydrolysed casein.

Lecthinase Activity

The isolated cultures such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus*, and *Bacillus subtilis* inoculated in egg yolk media after incubation the production of lecthinase is observed by cream white color colonies.

Enzyme Immobilization Technique

Production of extracellular crude lipases has been remarkable in ordinary growth media, for commercial production extracellular lipases can be stabilized in immobilization method in this preparation we used Sodium alginate as the carrier molecule, the enzyme beads formed can be preserved and it can be used for further application.

Significant and Impact of the Study

The present study was focused to isolate and characterize lipase producing bacteria from various samples. Different species of *Bacillus* such as *Bacillus pumilus, Bacillus subtilis, Bacillus megaterium, Bacillus cereus* and *Pseudomonas aeruginosa* was isolated and screened. All these lipase producers appeared promising good source of lipase but it requires a detail characterization of growth and nutrient conditions. The optimization process of lipase enzyme at various pH with different isolates was standard, and with different lipid sources was also reasonable in producing lipase. This enzyme is an need for development of detergents and soap oils in industrial sector so its efficacy is reliable on fermentation process.

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