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# Isolation and characterization of lipolytic *Pseudomonas spp.* from oil contaminated water samples

Vivek S. Vishwe[1]*, Shrikant P. Gajbhiye[1], Shashikant P. Vaidya[2], and Abhay S. Chowdhary

[1]*(Department of Bacteriology, Haffkine Institute for Training, Research and Testing, Parel, Mumbai 400 012, India)*

[2]*(Department of Clinical Pathology, Haffkine Institute for Training, Research and Testing, Parel, Mumbai 400 012, India)*

***Abstract:*** *Oil contaminated water samples collected from different areas in Pune were screened for the selective isolation of lipolytic Pseudomonas spp. Screening medium containing Tributyrin (1% v/v) and Rhodamine B was used for the specific isolation of lipolytic Pseudomonas spp. from oil contaminated water samples. Lipolytic bacteria showing zone of clearance around the colonies were selected for the present study. Isolated bacteria were identified upto genus level with the help of morphological and biochemical testing by following Bergey's manual. Spectrophotometric Lipase assay showed that out of 49 lipolytic Pseudomonas spp., WP23 exhibits the maximum Lipase activity of about 45.4733 U/ml.*

***Keywords:*** *Biochemical tests, Lipase, Rhodamine B, Spectrophotometric assay, Tributyrin.*

## I. Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are enzymes having a biological function of catalyzing the hydrolysis of triacylglycerols [1]. Lipase catalyzes the hydrolysis of triacylglycerols into a diacylglycerols, monoacylglycerols, glycerols and fatty acids at water-lipid interface [2,3]. In non-aqueous environment, Lipases can also catalyze ester synthesis [3,4]. Lipases are one of the most important enzymes that are used in various industries like Detergent, Food, Leather, Textiles, Pharmaceuticals, etc [4,5]. Bacterial Lipases are the enzymes with tremendous demand due to their potential industrial applications & stability. Although Lipase enzyme was isolated and purified from fungi, bacteria, yeast, animal and plant sources, but of all these bacterial Lipases are considerably commercially important and physiologically significant [6].

Among various Gram positive and Gram negative bacteria, the *Pseudomonas spp.* remain considerably less explored for the purpose of Lipase production. Hence the present study mainly focuses on the selective isolation of lipolytic *Pseudomonas spp.* from various oil contaminated water samples.

## II. Materials And Methods

### 2.1 Collection of oil contaminated water samples

Water samples mainly collected from oil contaminated sites like sewage treatment plants, riverside, bus stand-railway car shed, car-bike servicing and washing centers, etc. situated in and around Pune region were used in the present study for the isolation of lipolytic bacteria. Sterile containers were used for the collection of oil contaminated water samples. Then these samples were transferred to the laboratory for further analysis [7,8]. In the present study, total 14 oil contaminated water samples were collected and screened for the presence of lipolytic *Pseudomonas spp.*

### 2.2 Lipolytic bacteria isolation

Selective isolation of lipolytic bacteria from oil contaminated water samples were performed by using Tributyrin agar medium containing Rhodamine B. Pour plate method was used for the screening of water samples for isolation of lipolytic bacteria. Tributyrin Agar base medium containing Rhodamine B consists of components like tributyrin 1%(v/v), yeast extract 0.3%(w/v), peptic digest of animal tissue 0.5%(w/v), Rhodamine B 0.0001%(w/v) & agar 1.5%(w/v). Petriplates were then incubated for about 24 - 48 hours in an incubator at an incubation temperature of $37^0$C. After incubation, lipolytic bacterial colonies showing a clear zone of inhibition of tributyrin around the edges of the colonies were selected for the further studies [9,10,11,12].

### 2.3 Screening of lipolytic isolates

After completion of 24 - 48 hours of incubation, lipolytic bacteria which are Gram negative, rod shaped and motile in nature were selected for the present studies. Microbiological screening of isolated lipolytic bacteria was performed by four quadrant streaking method on Tributyrin agar plates containing Rhodamine B. These plates were then incubated at $37^0$C for 24 - 48 hours. After incubation, these plates were checked for the appearance of zone of inhibition around the bacterial colonies [13,14]. Isolated pure cultures of lipolytic bacteria were maintained in the form of glycerol stock preparations at $-20^0$C for purpose of preservation.

### 2.4 Biochemical characterization of the isolates

Identification of isolated lipolytic bacteria upto genus level was performed with the help of morphological and different biochemical characteristics. Gram's nature, bacterial colony characters, and motility of the organism were studied for morphological analysis while different tests like Indole test, Nitrate reduction test, Catalase test, Oxidase test along with carbohydrate / sugar fermentation were performed for analysis of biochemical characters of isolated lipolytic bacteria. With the help of morphological and biochemical characteristics along with Bergey's manual of Systematic Bacteriology, genus level identification of isolated lipolytic bacteria was performed [15,16].

### 2.5 Lipase assay

Extracellular Lipase activity was measured using polyoxyethylene sorbitan ester (Tween 80) as substrate by the method described by Tirunarayanan and Lundbeck with slight modifications. Tween 80 is the ester of oleic acid. Briefly, the reaction mixture contains 0.1ml of 10% Tween 80 in 50mM Tris hydrochloride buffer (pH 7.6), 0.5ml of concentrated culture supernatant as a source of enzyme, 0.1ml of 1M $CaCl_2$ in Tris buffer, and 2.3ml of Tris buffer (pH 7.6). Reaction mixture with 0.5ml of deionized water instead of supernatant was considered as a blank. Enzyme assay for each isolate was performed in triplicates. Then the reaction mixtures were incubated for 2 hours at $37^0$C in an incubator. In this spectrophotometric assay, Tween was cleaved to produce fatty acid and alcohol. Presence of calcium in the reaction mixture leads to the formation of an insoluble fatty acid salt, giving a precipitate which can be measured spectrophotometrically at 400nm. One unit of Lipase activity was defined as the amount of enzyme resulted in an increase of optical density at 400nm (OD400) of 0.01 after 2 hours under the assay conditions [17]. Lipase activity of all isolated lipolytic *Pseudomonas spp.* was determined and expressed in U/ml.

## III. Results

### 3.1 Screening, Isolation and Identification of the isolates

Total 49 (WP01-WP49) lipolytic *Pseudomonas spp.* were isolated from 14 oil contaminated water samples collected from different areas of Pune. All 49 lipolytic isolates were then identified upto genus level with the help of morphological and biochemical studies. All the lipolytic isolates were characterized as Gram negative, rod shaped, motile bacteria that are capable of hydrolysis of tributyrin **(Fig. 1)**. A zone of clearance around the bacterial colonies was observed due to the hydrolysis of tributyrin after 48 hours of incubation.

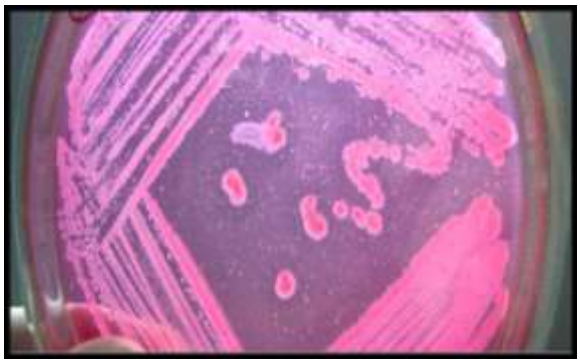

**Figure 1. Zone of clearance around the colonies due to hydrolysis of tributyrin on Tributyrin agar containing Rhodamine B.**

### 3.2 Lipase Assay

Enzyme assay for each isolate was performed in triplicates. After performing the Lipase assay of these 49 lipolytic isolates, it was found that WP23 exhibits maximum enzymatic activity **(Fig. 2)**. It was observed that WP23 possesses Lipase activity of about 45.4733 U/ml.

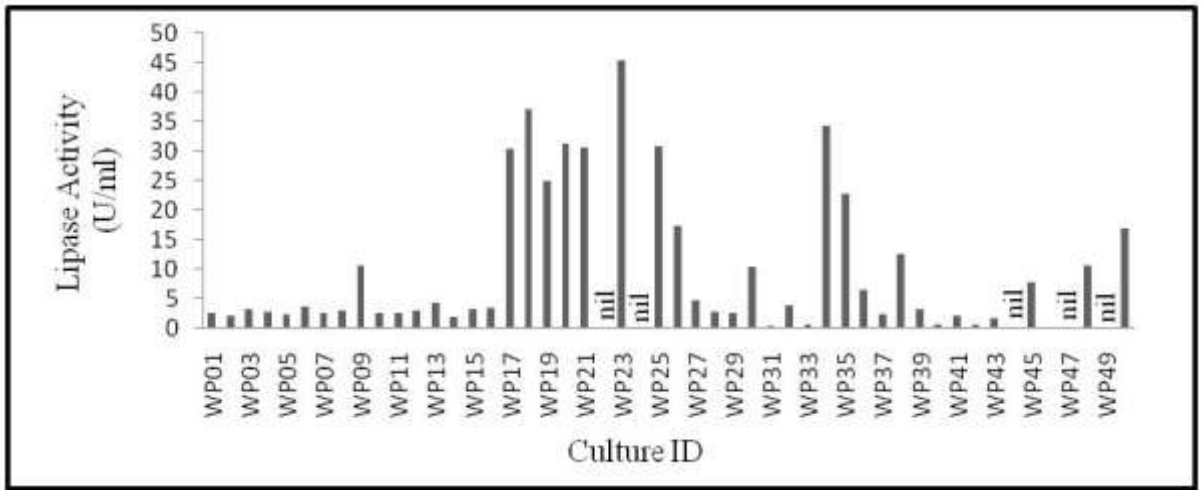


**Figure 2. Lipase activity of lipolytic *Pseudomonas spp.* isolated from water samples by spectrophotometric assay.**

### 3.3 Identification of the selected lipolytic strain

Morphological & biochemical characteristics of the lipolytic isolates were studied for the identification of the isolated bacteria. Lipolytic bacterial strain WP23 exhibit maximum Lipase activity as compared to the other strains as indicated in Figure 2. Morphological & biochemical studies of WP23 were carried out **(Table 1)**. According to the Bergey's manual of Systematic Bacteriology & 16SrDNA sequencing analysis, the isolate WP23 was identified and confirmed as *Pseudomonas aeruginosa*.

**Table 1: Morphological & Biochemical Characterization of WP23:**

| WP23 Colony Characters | Details | Biochemical Tests | Results |
|---|---|---|---|
| Shape | Circular | Gram Staining | Gram Negative Rods |
| Size | 2-3mm | Motility | Motile |
| Color | Creamy | Indole | Negative |
| Margin | Smooth | Catalase | Positive |
| Opacity | Opaque | Nitrate Reduction | Positive |
| Elevation | Slightly elevated | Oxidase | Positive |
| Consistency | Sticky | Sugar Fermentation | Glucose: Negative<br>Sucrose: Negative<br>Lactose: Negative<br>Maltose: Negative<br>Mannitol: Negative |
| Pigment | Green | | |

## IV. Discussion

Extracellular Lipase enzyme obtained from microbial origin possesses variety of applications in various industries. The present study helps us to understand the pre-existing industrial protocols for isolation and characterization of Lipase producing organisms. Lipolytic *Pseudomonas spp.* isolated from oil contaminated water samples possesses a high capability of extracellular Lipase production.

Out of 49 isolates (WP01-WP49), WP23 exhibits optimum Lipase activity. By using Bergey's manual of systemic Bacteriology and 16SrDNA sequencing analysis, WP23 was confirmed as *Pseudomonas aeruginosa*. The present study also reveals the fact that every lipolytic organisms isolated from oil contaminated water sample shows a wide diversity in the enzymatic activities.

## V. Conclusion

In conclusion, a total of 49 lipolytic *Pseudomonas spp.* were isolated after screening 14 oil contaminated water samples collected from different areas in Pune. After performing the spectrophotometric Lipase assay, it was found that WP23 showed Lipase activity of about 45.4733 U/ml. As WP23 exhibits optimum Lipase activity amongst them, WP23 was further identified and characterized upto species level with the help of Bergey's manual of systemic Bacteriology and 16SrDNA sequencing analysis. According to morphological and biochemical testing, WP23 was identified as *Pseudomonas aeruginosa*. WP23 can be effectively used for the extracellular Lipase enzyme production with the help of submerged fermentation. Optimization of fermentation parameters shall also play a crucial role in the enhanced production of Lipase enzyme.

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