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Characterization of extracellular lipase producing bacteria RV22 isolated from oil contaminated soil samples of Karnataka, India

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Abstract: **Bacteria produce lipases efficiently, having a wide range of applications in different industries. In the present study, lipase producing bacteria were isolated from different oil contaminated soil samples collected from Karnataka, India. Lipase producing bacteria were initially screened on tributyrate agar media and confirmed on Rhodamine B olive oil agar media. Among 35 lipase producing bacterial isolates, the potential isolate RV22 was subjected to biochemical characterization. And the bacterial strain RV22 identified as *Serratia* sp.**

Keywords: **Lipase, *Serratia* sp., optimization, Rhodamine B, fluorescence.**

I. INTRODUCTION

Lipases (3.1.1.3) are lipid or fat hydrolysing enzymes, they acts at lipid water interface catalysing the hydrolysis of triacylglycerols to yield glycerol and free fatty acids in aqueous media [1]. Many lipase producing bacterial species belonging to the genera *Serratia* [2], *Acinetobacter* [3], *Bacillus* [4], *Pseudomonas* [5] etc., have been reported. Bacteria produce lipases efficiently, having wide range of applications in the field of dairy [6], pharmaceuticals [7], food industries [8], detergent formulations [9] etc. Lipase biosensors are used to detect pesticides in water bodies [10]**.** Different qualitative and quantitative assays are in use for detection of microbial lipases [11], [12]. In Indian scenario many researchers focused on lipase producing bacteria [13], [14]. In the present study, lipase producing bacteria were isolated from different oil contaminated soil samples of Karnataka, India. Preliminary screening for lipase producing bacteria was performed on TBA then confirmed on Rhodamine B olive oil agar media (RBA). The potential selected bacterial isolate RV22 was subjected to biochemical characterization and identified as *Serratia* sp.

II. MATERIALS AND METHODS

A. Sample Collection: Oil contaminated soil samples were collected from different regions of Kalburgi District, Karnataka, India. Soil samples were collected aseptically using sterile spatula 4-5 cm below the ground level in sterile containers and transported to the laboratory and stored at 4 ºC for further use.

B. Preparation of soil suspensions and screening for lipolytic bacteria: Soil suspensions were prepared by adding 1g of each soil sample to 100ml sterile water in sterile flasks and incubated at 37 ºC in a rotary shaker at 120 rpm for one hr. After one hr incubation serially diluted the obtained suspensions to get dilutions up to 10^{-7}. Preliminary screening was performed by inoculating 0.1 ml aliquots of 10^{-7} dilution of soil suspension by pour plate method on TBA of pH 7.5 containing peptone 5 g, beef extract 3g, tributyrin 10ml and agar-agar 20g, distilled water 1000ml and observing clear zone forming colonies after incubation for 48 hrs at 37 ºC. Clear zone forming colonies on TBA were selected and confirmed by inoculating on RBA and by observing orange fluorescence halo around the colonies under UV radiation [15]. Confirmation test is necessary since esterase producing bacterial isolates also form clear zone around the colonies on TBA [16].

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C. Selection of potential isolate for biochemical characterization: The positive lipase producers confirmed on RBA were selected and inoculated on to production media [17]. After incubation, the cell free extract containing the crude enzyme was used to determine potential lipase producers by agar well diffusion assay, a modified method of [18]

D. Biochemical characterization: Selected potential isolate RV22 isolate was subjected to biochemical characterization, by performing different morphological and biochemical tests [19] [20].

III. RESULTS AND DISCUSSION

In the present study we collected 3 oil contaminated soil samples from Kalburgi district of Karnataka, India. Soil contains a wide range of bacteria helpful in various ways [21], [22]. Qualitative determination of lipase production was done on TBA and RBA. There are total 270 numbers of bacteria were isolated from oil contaminated soils, all these were preliminarily screened on TBA and confirmed RBA, for lipase production. Among the 270 isolates, 35 isolates showed zone of lipolysis on both media. Among the 35 isolates 3 isolates showed maximum lipolytic activity. Further maximum lipolytic activity showed (i.e. zone of lipolysis on Rhodamine B agar well is 40mm well) isolate RV22 was subjected to biochemical characterization.

Previously there are several researchers reported lipolytic bacteria from oil contaminated regions/soil [23], [24]. The lipolytic activity has been the potentiality of the bacteria to survive in the oil contaminated region, by utilising oil as its carbon source. The TBA and RBA medium widely used for qualitative and quantitative determination of lipolysis by bacteria. In the present study we are also using both TBA and RBA media for determination of lipolysis by bacteria, and results were in line with other researchers. Biochemical tests were done for bacteria. The isolate RV22 showed different biochemical tests which were tabulated in Table 1. Based on the morphological and biochemical analysis, isolate RV22 confirmed as *Serratia* sp. *Serratia* sp. is facultative anaerobe Gram -Ve motile rod. There are many researchers reported the lipolysis activity or lipase production from *Serratia* [25], [26]. In many cases, the authors used either TBA or RBA for screening of lipolytic organisms. In present study both TBA and RBA were employed for preliminary screening and conformation of lipolytic bacteria, respectively. And a modified method of [18] plate assay is used to select the potent lipase producers. Zone of the lipolysis of crude enzyme extract of RV22 was found ~40mm on Rhodamine B agar wells.

Table I: Biochemical and Morphological characterization of the isolate RV22

Sl no.	Morphological and biochemical characteristics	RV22
A.	**Colony morphology**	
1.	Margin	Entire/round
2.	Elevation	+
3.	Color	Red
4.	Texture	Smooth
B.	**Cell morphology and Biochemical characteristics**	
1.	Shape	Rods
2.	Arrangement	Single
3.	Motility	+
4.	Grams Reaction	_
5.	Catalase	+
6.	Oxidase	_
7.	Indole	_
8.	MR	+
9.	VP	+
10.	Citrate	+
11.	Urease	_
12.	Gelatinase	+
13.	H2S	_
14.	Nitrate red	+
15.	Starch	_
16.	Glucose	+
17.	Lactose	_
18.	Sucrose	+
19.	Gas	_
20.	Mannitol	+

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