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Isolation and Characterization of Vegetable Oil Degrading Bacteria

P. Prajakta Kamble*, A. Avinash Raut and M.B. Gandhi

PG Department of Microbiology, Yashwantrao Chavan College of Science, Vidyanagar, Karad - 415 124, India.

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For the present study the suitable vegetable oil contaminated samples were collected from Kolhapur and Karad cities, in India. Four quadrant streak plate technique was used for the isolation and characterization of efficient vegetable oil degrading strains that had ability to grow on 1% vegetable oil concentration on Bushnell and Hass agar plates. These isolated strains namely $KopB_1$ and $KRDB_1$ were identified by morphological and biological tests and were found to belong to Genus: *Staphylococcus* and *Bacillus*. They were also able to grow up to 7 and 8% vegetable oil concentration respectively.

Key words: Oil contamination, Edible oil, Microorganisms.

Oils, fats and waxes have been in use by man since very ancient times. The oils derived from plants usually extracted from seeds are important products used pharmaceutically, industrially and nutritionally[1].

Many vegetable oils are used directly as ingredients in food as it has various properties like…

*High caloric value

* It makes the ingredient stick less to each other

* It can also carry flavours to other ingredients in food[2]. In pharmaceutical industries vegetable oils are used for preparing concentrated solutions of vitamin A and D. The groundnut oil emulsion has been used successfully for the control of many insect pests of plants. Many vegetable oils are used to make soaps candles, perfumes, cosmetic products, insulators, biodiesel etc.[3]. Thus production, use and transportation of vegetable oils is growing fast around the world. At the same time the frequency of chocking up of sewage lines because of oily wastes of vegetable origin generated from house, canteens, armybases, restaurants, pharmaceutical industries and various other industries, has been increased resulting in foul smell and back up problems. Hence there is a need for microorganisms capable of degrading vegetable oil wastes[4].

The aim of this study was to screen the efficient bacteria capable of utilizing vegetable oil as the sole 'C' and energy source that can be used for purifying oil contaminated effluents.

MATERIAL AND METHODS

Collection of samples

Two oil contaminated soil samples were collected constituting filtrate obtained after oil extraction from Shree Oil Mills, Kolhapur and oily soil from Vadapav centre, near Yashwantrao Chavan College of science, Karad.

These samples were tested for the presence of vegetable oil degrading bacteria.

Isolation

Isolation of vegetable oil degrading organism was carried out using four quadrant streak plate method on Bushnell and Hass medium[5]. Containing vegetable oil as the sole 'C' and energy source. Plates were incubated at room temperature for 6 days. Bacterial colonies after Gram staining were transferred on Nutrient agar and incubated at room temperature for 24 hrs. Slants of pure cultures were maintained at 4°C.

* To whom all correspondence should be addressed.
Mob.: +91-9403576390
E-mail:prajakta14@rocketmail.com

Table 1. Collection of Samples and Coding of Isolation

Samplecode	Source of sample	IsolatedCode
Kop	Filtrate after oil extraction, Shree Oil Mills, Kolhapur	$KopB_1$
KRD	Oily Soil from Vadapav Centre, near Yashwantrao Chavan college of Science,Karad	$KRDB_1$

Table 2. Colony Morphology of Bacterial isolates

Isolate Code	Size (mm)	Shape	Colour	Margin	Elevation	Opacity	Consistency
$KopB_1$	1	circular	Goldenyellow	Entire	LowConvex	Opaque	Moist
$KRDB_1$	2	circular	White	Entire	LowConvex	Opaque	Moist

Table 3. Biochemical Characteristic of bacterial isolates

Characters	Isolates	
	$KopB_1$	$KRDB_1$
Gram Staining	Gm +ve cocciin cluster	Gm +ve rodsin chains
Motility	non-motile	Sluggishly motile
Spore formation	-	oval central spore
H_2S production	-	-
Nitrate reduction test	+	+
Catalase	+	+
Oxidase	-	+
Urea hydrolysis	+	-
Starch hydrolysis	-	+
Gelatin hydrolysis	+	+
Caseinase	+	+
Lipase	+	+
Chitinase	-	+
Pectinase	-	-
Cellulase	-	+
Carbohydrate fermentation		
Glucose	+	-
Lactose	+	-
Mannitol	+	-
Mannose	+	+
Maltose	+	+
Galactose	+	-
Sucrose	+	-
Fructose	+	-
Rhamnose	-	+
Xylose	+	+

+ = positive test; - = negative test

Table 4. Physiological characteristics

Table 4.1. Effect of temperature on growth of isolates

Isolate Code	Temperature					
	4°C	15°C	28°C	37°C	45°C	55°C
$KopB_1$	-	+	+++	+++	++	-
$KRDB_1$	-	-	+++	+++	++	+

+++ = Rich growth; ++ = Good growth; + = Growth; - = No growth

Table 4.2. Effect of pH on growth of isolates

Isolate	pH				
Code	3	5	7	9	11
$KopB_1$	-	+	+++	+	-
$KRDB_1$	-	+	+++	+	-

+++ = Rich growth; ++ = Good growth;
+ = Growth; - = No growth

Table 4.3. Effect of salt concentrations on growth of isolates

Salt Conc. %	Isolates	
	$KopB_1$	$KRDB_1$
1	+++	+++
2	+++	+++
3	+++	+++
4	++	++
5	++	++
6	++	++
7	++	++
8	+	-
9	+	-
10	+	-
11	+	-
12	+	-
13	+	-
14	+	-
15	+	-
16	+	-
17	+	-
18	+	-
19	-	-
20	-	-

+++ = Rich growth; ++ = Good growth; + = Growth; -

Characterization of isolates

Examination of isolates for its size, shape, margin, consistency, opacity, elevation, Gram nature and motility[6] was carried out. The isolates were characterized as described by Sneath *et al*.,[7]. Biochemical properties used includes, production of catalase, oxidase, urease, caseinase, lipase, chitinase, pectinase, cellulase, oxidative fermentation of sugar, nitrate reduction, hydrogen sulphide production, gelatin liquefaction and starch hydrolysis.

Physiological characterization of isolates

Effect of various temperatures, pH, NaCl concentrations up to (20%) and vegetable oil concentrations up to (8%) on the growth of isolates was studied.

RESULTS AND DISCUSSION

Two bacterial cultures were isolated from vegetable oil contaminated samples by four quadrant streak plate technique. According to morphological, biological and physiological characteristics, cultures namely $KopB_1$ and $KRDB_1$ seem to belong to Genus: *Staphylococcus* and *Bacillus*.

The *Staphylococcus spp*. ($KopB_1$) showed growth at 15^0C,28^0C,37^0C and 45^0C and showed maximum tolerance up to 18% to salt concentration and up to 7% to vegetable oil concentration.

The *Bacillus spp.* ($KRDB_1$) was able to produce chitinase and cellulase after 2 days incubation. It showed growth at 28^0C, 37^0C, 45^0C and 55^0C and can tolerate salt concentration up to 7% and showed growth up to 8% vegetable oil concentration.

Table 4.4. Effect of vegetable oil concentrations on growth of isolates

Isolate Code	Vegetable Oil Concentration%							
	1	2	3	4	5	6	7	8
KopB1	+++	+++	+++	+	+	+	+	-
$KRDB_1$	+++	+++	+++	++	++	++	+	+

+++ = Rich growth; ++ = Good growth; + = Growth; - = No growth

CONCLUSION

Two bacterial strains namely $KopB_1$ and $KRDB_1$ seem to belong to Genus: *Staphylococcus* and *Bacillus* were isolated from two samples contaminated by vegetable oil collected from Kolhapur and Karad cities. Isolation was carried out using Bushnell and Hass medium containing vegetable oil as sole source of carbon.

These bacterial isolates from vegetable oil contaminated sites are able to utilize the vegetable oils namely soybean oil, cottonseed oil and groundnut oil for growth. As a response to biodegradation these cultures may be used to actively degrade the pollutants and detoxify the environment. This will help in solving the clogging and back up problem of sewer lines[8].

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