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Isolation and Characterization of Vegetable Oil Degrading Yeasts

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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 $KopY_1$ and $KRDY_1$ were identified by morphological and biological tests and were found to belong to Genus: *Saccharomyces*. They were also able to grow on media containing up to 8% vegetable oil concentration.

Key words: Oil contaminated samples, Vegetable oil, Yeasts.

Vegetable oils usually extracted from seeds are used by man from ancient time on large scale at household as well as at industrial level important products used pharmaceutically, industrially and nutritionally.

These oils have 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; hence they are directly used as ingredients in food[5].Vegetable oils are used for preparing concentrated solutions of vitamin A and D in pharmaceutical industries. The groundnut oil emulsion has been used successfully to control many insect pests of plants. Many vegetable oils are used to make soaps, candles, perfumes, cosmetic products, insulators, biodiesel etc. [7]. So the production, use and transportation of vegetable oils is rapidly growing around the world.

The oily wastes of vegetable origin generated from house, canteens, army bases, restaurants, pharmaceutical industries and various other industries contribute their fair share towards environmental pollution[8] as these waste products are responsible for clogging sewer networks, grease trap formation and accumulation of toxic substances in environment contaminating public water bodies and adversely affecting human health[9].

Hence the aim of this study was to screen the efficient yeast capable of utilizing vegetable oil as the sole 'C' and energy source that can be used to solve the problem caused due to accumulation and putrefaction of the oils.

MATERIAL AND METHODS

Collection of samples

Two oily samples were collected soil near oil mill machine Shree Oil Mills, Kolhapur and oily scrapping around oil lamp in Hanuman Mandir, Banawadi Road, Karad.

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

Isolation

Isolation of vegetable oil degrading organism was performed on Bushnell and Hass medium containing vegetable oil as the sole 'C' and energy source, using four quadrant streak plate method. Plates were incubated at room temperature for 6 days. Yeast colonies after Gram staining were transferred on Glucose Yeast Extract agar and

Table 1. Collection of Samples and Coding of Isolation

Samplecode	Source of sample	IsolatedCode
Kop	Soil near Oil Mill Machine, Shree Oil Mills, Kolhapur.	$KopY_1$
KRD	Oily Scrapping around oil lamp in Hanuman Mandir, Banawadi Road, Karad.	$KRDY_1$

Table 2. Colony Morphology of Yeast isolates

IsolateCode	Size(mm)	Shape	Colour	Margin	Elevation	Opacity	Consistency
$KopY_1$	2	circular	White	Serrate	LowConvex	Opaque	Moist
$KRDY_1$	1	circular	White	Entire	LowConvex	Opaque	Sticky

Table 3. Biochemical Characteristic of yeast isolates

Characters	Isolates	
	$KopY_1$	$KRDY_1$
.Gram Nature	Gram positive	Gram positive
.Shape of the cell	Ellipsoidal	Elongated
.Budding/Fission	Budding	Budding
.Pellicle formation	+	+
.Acid production	+	+
.Osmophilicity	+	+
.H_2S production	+	+
.Nitrate reduction test	+	+
.Catalase	+	+
.Oxidase	+	+
.Urea hydrolysis	-	-
.Starch hydrolysis	-	+
.Gelatin hydrolysis	-	+
.Caseinase	-	+
.Lipase	+	+
.Chitinase	-	-
.Pectinase	+	-
.Cellulase	+	+
.Carbohydrate fermentation		
Glucose	+	+
Lactose	+	-
Sucrose	+	+
Melibiose	+	+
Maltose	+	+
Galactose	+	+
Raffinose	-	+

+ = 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
$KopY_1$	-	-	+++	+++	++	+
$KRDY_1$	-	-	+++	+++	+	+

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

Table 4.2. Effect of pH on growth of isolates

Isolate Code	pH				
	3	5	7	9	11
$KopY_1$	+	+	+++	+	+
$KRDY_1$	+	+	+++	+	+

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

Table 4.3. Effect of salt concentrations on growth of isolates

Salt Concentration %	Isolates	
	$KopY_1$	$KRDY_1$
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; - = No growth

incubated at room temperature for 24 hrs. Slants of pure cultures were maintained at 4°C.

Characterization of isolates

Identification and Characterization of yeast isolates was performed using colony characters, Gram nature, budding/fission mechanism, acid production, pellicle formation and Osmophilicity. For biochemical characterization of isolates following tests were performed as described by Gibbs and Salle like catalase, oxidase, urease, caseinase, lipase, chitinase, pectinase, cellulase production, sugar fermentation, 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 yeast cultures were isolated from vegetable oil contaminated samples by four quadrant streak plate technique. According to morphological, biological and physiological characteristics, cultures namely $KopY_1$ and $KRDY_1$ seem to belong to Genus: *Saccharomyces*.

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
KopY1	+++	+++	+++	+	+	+	+	+
$KRDY_1$	+++	+++	+++	++	++	++	+	+

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

The isolates namely $KopY_1$ and $KRDY_1$ have the ability to grow on vegetable oil concentration up to 8% and temperature up to 55^0C. They also tolerated acidic as well as alkaline pH. Both were osmophilic and produced cellulase enzyme.

Isolate $KopY_1$ also produced pectinase and could tolerate salt concentration up to 9% .Isolate $KRDY_1$ showed growth up to 11% salt concentration.

CONCLUSION

Two yeast strains namely KopY1 and $KRDY_1$ seem to belong to Genus: *Saccharomyces* 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.

Both the isolates from vegetable oil contaminated sites are able to utilize the vegetable oils namely soybean oil, cottonseed oil and groundnut oil up to 8% for growth. They were osmophilic, produced cellulase and could tolerate high temperature and extreme pH conditions. Thus, these cultures may be used for actively treating vegetable oil contaminated wastes. This will help in solving the clogging and back up problem of sewer lines.

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