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BIODEGRADATION OF CRUDE OIL BY USING *SACCHAROMYCES CEREVISIAE* ISOLATED FROM PALM WINE

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ABSTRACT: Diverse microbial population's bacteria, yeast, or molds have been reported for degradation of hydrocarbons. The reported efficiency of biodegradation ranged from 6% to 82% for fungi. So that the study of isolation of *Saccharomyces cerevisiae* from palm wine sample collected from various areas in Cuddalore district. Two sample of palm wine were collected. The palm wine was collected from Chidambaram and Neyveli. The samples was serially diluted and perform the spread plate technique. The plates are incubated and colonies are counted (CFU/ml). Perform the biochemical test for identification of *Saccharomyces cerevisiae* from the counted plate colonies. Further prepare the pure culture of *Saccharomyces cerevisiae* in YPGB(Yeast extract peptone glucose broth) incubate at 25^0C for 72 hours. Prepare the MSM (Mineral Salt Medium) for biodegradation process of crude oil. Crude oil collected from the local area (Neyveli). 1ml of crude oil added into MSM media and inoculate the pure culture ofthe *Saccharomyces cerevisiae* it is a sample flask. The biodegradation process was done in twice (one for sample and another one for control).The control flask contain only MSM and 1ml of crude oil withoutmicroorganism. The conical flask are incubated at 28^0C for 28 days. Finally, noted the biodegradation ability of *Saccharomyces cerevisiae*. In the present was mainly focus to isolate the *Saccharomyces cerevisiae* from palm wine and isolated strain were used to determine the biodegradation of crude oil.

Key words: **Palm wine, Crude oil, Biodegradation, *Saccharomyces cerevisiae*.**

INTRODUCTION

Cultures of microorganisms used for the production of alcoholic beverages and fermented foods all over the world. Even through the scientific explanation and the identification of these beneficial microorganisms mostly lactic acid bacteria, filamentous moulds and yeasts were unknown to people in the past, they cultured them traditionally for production of foods. In our India fermented foods are prepared exclusively using bacteria or bacteria yeast mixed culture. For example we prepare fermented food like curd and idly making flour, these are well knownabout yeast packing foods in our early day today life (Danmadami *et al.*,2017).

Palm wine or kallu (in Tamil) in an alcoholic beverage produced from the sap of various species of palm tree such as palmyra. This is commonly called Panai maram (scientific name *Borassus flabellifer*) in tamilnadu. This is state tree of tamilnadu. It's the cultural and also traditional tree to mingle the Tamilian people. Palm wine maybe distilled to produce a strong drink "Kallu". The sap of the palm trees in originally sweet and serves as a rich substrate for the growth of various types of microorganisms (Amoa-Awua *et al.*,2007&Naknean *et al.*,2010).The sap undergoes spontaneous or wild fermentation which promotes the proliferation of yeasts and bacteria that bring about the conversion of the sweet substrate into several metabolites such as ethanol, lactic acid and acetic acid. Yeasts, lactic acid bacteria and acetic

acid bacteria play the most important roles in the palm wine production (Ouoba *et al*., 2012).

The utilization of isolated strains of *Saccharomyces cerevisiae* is an important strategy for keeping the quality and assuring the reproducibility of wine features. The utilization of strains isolated from specific regions could be evenmore interesting because of their high adaptation to their own climatic conditions and grapes.

Even more, these strains are usually associated to particular wine characteristics that frequently identify specific wines and regions. Thus, the aims of this work is to isolate and characterize *Saccharomyces cerevisiae* strains from palmwine (Revista brasileira de ciências farmacêuticas *et al.*, 2006).

Crude oil is a natural product, comprising a complex mixture of various hydrocarbons, created by the decomposition of plant remains from the carboniferous period under high temperature and pressure (Van Hamme *et al.*, 2003). The broadest environmental pollutants classification is into two major categories: organic and inorganic pollutants (Jim *et al.*, 2005).

Quantitatively organic pollutants that are of most concern are the hydrocarbons in their various forms.The most common are petroleum hydrocarbons (mixtures of n-alkanes, mono-, di-and polyaromatic compounds,heterocyclic aromatics and other minor constituents) and host of other compounds (Jim *et al.*, 2005).The petroleum industry is a major contributor of organic contaminants to the natural environment, releasing hydrocarbon contaminants into the environment in anumber of ways.Accidental and deliberate crude oil spills have been and still continue to be, a significant source of environmental pollution and poses a serious environmental problem, due to the possibility of air, water and soil contamination (Trindade *et al.*, 2005).

There is also food chain disruption following the loss of phytoplankton,shellfish, fish and birds. Microbial degradation of crude oil as a means of clearing oil spills in the natural environment is a slow process and therefore, stimulated biodegradation through microbial seeding, application of fertilizer, tilling and liming (if the soil is acidic) or a combination of all these methods may be the answer (Isinguzo and Odu, 1987; Ekundayo and Obire, 1987).

MATERIALS AND METHODS

The palm wine sample were collected from our local area (Chidambaram and Neyveli). Crude oil was collected from mechanic shop in Neyveli.

Chemicals: The chemicals and reagents used in the present investigation was Rose Bengal agar, Yeast extract peptone broth, Mineral Salt Medium, Nutrient Broth, Gram staining, Glucose and Sucrose broth. The glassware's used in the present study were conical flask of 100ml and 250ml, test tubes, Petri plates.

Isolation of *Saccharomyces cerevisiae*

Serial dilution: This method used to reduced population of microorganism in palm wine.

Procedure: Six test tube was taken in the tube stand. Each test tube contain 9ml distilled water test tube denoted into10^{-1}, 10^{-2}, 10^{-3},10^{-4},10^{-5} and10^{-6}. Add 1ml of palm wine in first tube by using pipette then it continuously transferred 1ml into further test tube. Finally, 1ml was discarded (O.P Abioye *et al*., 2013).

Spread plate technique: The diluted sample was plated by spread plate method.

Procedure: Prepare 80ml of Rose Bengal agar for four Petri plate. The prepared RBA is sterilized and cooled then poured into sterilized petridishes. After solidification, the spread plate was done.$10^{-3,}10^{-4}$ and 10^{-5} factors are used for spread plate technique.0.1ml was taken from each tube added into plates spread by using L-rod. Another one plate used as control. The plate was incubated at 25^{0}C for72 hour (O.P Abioye *et al*., 2013). After incubation, colonies are counted and calculate the CFU (colony forming unit)/ml.

Calculation

$$\text{Colony forming unit} = \frac{\text{Colonies count} \times \text{Dilution factor}}{\text{Volume plated of sample}}$$

Identification of *Saccharomyces cerevisiae*

Gram staining: Gram staining mainly used to differentiate the cells depend upon themorphology and color of staining (Tambuwal *et al.*, 2018).

Procedure: Take a clean, grease free slide. Prepare the smear of suspension on the cleanslide with a loop full of sample. Air dry and heat fix Crystal violet was added into the smear wait for 60 seconds wash with water. Flood the gram's iodine for 1 minute and wash with water. Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water. Add safranin for about 1 minute and washwith water. Air dry, blot dry and observe under Microscope.

Conformation test

Ethanol tolerance test: The capability to tolerate various stresses (osmolarity and ethanol) is one of the main criteria to select strains for efficient ethanol fermentation. The commonchallenge of strains can be overcome by using ethanol tolerant yeasts and hence the selection of strain with ethanol tolerance is important. To evaluate the ethanol tolerance capacity, the isolated strains were inoculated in the TGY medium containing different concentration of ethanol (5, 7, 9, 10, 12, 14, 16 and 20% (v/v))(Thais *et al.*, 2006).

Procedure

Three clean glass test tube was taken. Ethanol was taken in three different concentration 10%, 12% and 14% added into the three tubes. Add 0.1 ml of isolate in each test tube and incubate at 30^0C for 72 hours.

Triple Sugar Iron test: The triple sugar-iron agar test employing Triple Sugar Iron Agar is designed to differentiate among organisms base done the differences in carbohydrate fermentation patterns and hydrogen sulfide production. To determine the ability of an organism produce the hydrogen sulfide.

Procedure

Prepare TSI agar and make a agar slant. Touch the top of a well-isolated colony by using inoculation loop. Inoculate TSI by first stabbing through the agar slant in the tube and then streaking the surface of the agar slant. Leave the cap on loosely and incubate at 25°-37°C in ambient air for 18 to 24 hours.

Carbohydrate fermentation test: Carbohydrate fermentation is indicated by the production of gas inside the Durham's tube. Some microbes are able to produce the gas obtained the carbohydrate sources like glucose, sucrose, etc. The Durham's tube insert inside the test tube containing Glucose broth and Sucrose broth then inoculate the isolate into the tube and incubate at 30^0C for 24 hours. (O.P Abioye *et al.*, 2013).

Temperature tolerance test: The isolate were plated in YPG and incubated at 25, 30, 37 and 45^0C for 72 hours (Thais *et al.*, 2006).

Flocculation test :The isolates were inoculated in 10ml liquid YPG and incubated at 30^0C for **72** hours. After incubation, the tubes were agitated for the visualization of flocculation (Thais *et al.*, 2006).

Hydrogen sulfide production: It is more sensitive method than TSI method. This method used to detect theH_2S production. The isolates were plated in LA and also in YPG and incubated 30^0C for 10 days (Thais *et al.*, 2006).

Pure culture preparation of *Saccharomyces cerevisiae*

Yeast peptone glucose broth [YPGB] used for pure culture preparation of *Saccharomyces cerevisiae.* 50ml of YPGB was prepared and sterilized at 121^0C. After sterilization, the colonies of *Saccharomyces cerevisiae* were inoculated into the YPGB using inoculation loop. It incubate at 25^0C for 48 hours. Pure culturealso prepared in nutrient broth (Thais *et al*., 2006).

Biodegradation of crude oil by *Saccharomyces cerevisiae*

Media preparation and inoculation process: Two conical flask was taken. 100ml of MSM media was prepared in the each conical flasks (both flask contain 50ml). 1ml of crude oil was added into the each conical flask. 1ml of broth culture of yeast isolate was added in the one conical flask and another conical flask taken as control. Both flask are incubate at 30^0C for 28 days. The result was observed at regular intervals (seven days intervals) and noted (O.P Abioye *et al.*, 2013).

Biodegradation studies with yeast isolates: The biodegradation studies was carried out by inoculating 1ml of 24 hour broth culture of the yeast isolate into 50ml of sterile Mineral Salt Medium [MSM] that contained 1g of crude oil in a Erlenmeyer flask. The experiment was set up in triplicate with control flasks which contained 50ml of sterile mineral salt medium plus 1g of crude oil but without added microorganisms. The flasks were incubated at 30^0C for 28 days. At seven days intervals, duplicate flasks per organisms plus control flasks were removed from incubator and the residual crude oil extracted with 20ml of diethyl ether and dried with anhydrous sodium sulphate. The solvent was evaporated using rotary evaporator and the weight of the residual oil was measured and recorded. The percentage biodegradation of the crude oil was calculated using the formula of Ljah and Ukpe [1992] as stated below.

Biodegradation [%] = weight of oil [control] - weight of oil [degraded]/weight of oil [control]×100

RESULTS

Spread plate technique: The colonies are grown in the plates. The fluffy, smooth, moist, glistening and cream color colonies was observed. Colony forming unit was calculated.

Gram staining: Purple color, oval shaped cells are observed under microscope.

Ethanol tolerance test: *Saccharomyces cerevisiae* have ability to tolerate the ethanol concentration.

The isolates produce negative result inside the tube.

Triple sugar ion test: The isolates produce gas and H_2S. Inside the tube isolates produce black and yellow color in TSI agar.

Carbohydrate fermentation test: The isolates produce gas inside the Durhum's tube and acid also producedinside the tube.

Temperature tolerance test: The isolates can able to tolerate the temperature $25^0C - 45^0C$.

Flocculation test: After incubation, the tubes were agitated for the visualization of flocculation.

Hydrogen sulfide test: The isolates produce black precipitate in the tube that indicates the H_2Sproduction in tube.

Biodegradation of crude oil calculation: Biodegradation of crude oil result were note at regular intervals (7 days).Calculate the percentage of degrade crude oil level compared to uninoculated flask.

DISCUSSION

The yeast isolate used in this study was identified as *Saccharomyces cerevisiae*. The microorganism has been previously reported to be found and isolated from oil polluted sites by Obire (1998) who also reported that fungi suchas *Saccharomyces, Rhodotorula, Sporobolomyces* have evolved with the ability to degrade petroleum unlike other groups of microorganisms.

The results of the percentage biodegradation of crude oil by *Saccharomyces cerevisiae* increases with increase in incubation period from 7 to 28 days thisshows the potential of *Saccharomyces cerevisiae* to use crude oil as a sole carbon source.

This might be us result of efficient hydrocarbon uptake via special receptor sites for binding hydrocarbons and might have a unique feature that assist in the emulsification and transport of hydrocarbons into the cell and also presence of enzyme that introduce molecular oxygen into the carbon and generate intermediates that subsequently enter common energy yielding catabolic pathway.

Naphthalene was converted to pentadecane, the result agreed with the report of Muncnerova and Augustin(1994) that *Saccharomyces cerevisiae* has the ability to oxidise or transform polycyclic aromatic hydrocarbons and render them non- toxic. The n-alkane such as Nonadecane, Eicosane, Docosane Heneicosane and Tetracosane were completely degraded. This is in agreement with the review of Obuekwe (2005) and Ashraf and Ali (2006) on the utilization of n-alkanes by yeastas a sole carbon and energy source.

CONCLUSION

Saccharomyces cerevisiae isolated from fermented palm wine has the potential to utilize crude oil as sole carbon source. The components degraded from the crude oil were majorly the n-alkane. Some of the aromatic hydrocarbons were converted into an intermediate product. *Saccharomyces cerevisiae* could be a good candidate microorganism in the bioremediation of crude oil contaminated sites because of its environmental friendly.

TABLE 1
COUNTING OF COLONIES IN SPREAD PLATE BY COLONY FORMINGUNI[CFU]

S.no	Palm wine	Total no of colonies CFU/ml		
		10^{-3}	10^{-4}	10^{-5}
1.	Sample 1	35×10^{-1}	3×10^{-2}	27×10^{-1}
2.	Sample 2	8×10^{-1}	29×10^{-2}	3×10^{-3}

The results in table 1 shows the calculation of colony forming unit in spread plates.

TABLE 2
IDENTIFICATION OF *SACCHAROMYCES CEREVISIAE*

S.no	Test name	Result
1.	Gram staining	Purple, oval cells
2.	Ethanol tolerance	Negative (more than 10%)
3.	Triple sugar iron	Acid and Gas
4.	Carbohydrate fermentation test	Gas production
5.	Temperature test	Positive
6.	Flocculation test	Positive

7.	Hydrogen sulfide	Positive

The results in table 2 shows the results of identification test for *Saccharomyces cerevisiae.*

TABLE 3
PERCENTAGE OF CRUDE OIL BIODEGRADATION BY *SACCHAROMYCES CEREVISIAE*
OIL BIODEGRADATION (%)

Microbial isolate(days)	**7**	**14**	**21**	**28**
Saccharomyces Cerevisiae	15.45 ± 1.7	24.69 ± 1.3	5.1 ± 14.	49.29 ± 5.0
Control	1.6 ± 0.8	2.3 ± 1.20	1.90 ± 0.9	2.50 ± 1.4

The results in table 3 shows the percentage of crude oil degradation by *Saccharomyces cerevisiae*. At the end of 28 days, 49.29% oil biodegradation was recorded by the test organisms compared to 2.5% biodegradation recorded in the uninoculated control

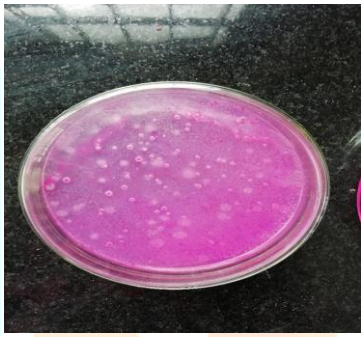

Figure 1: Isolation of *Saccharomyces cerevisiae* from palm wine by spread plate

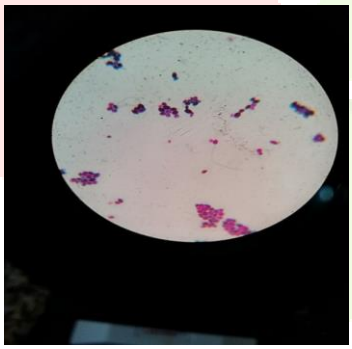

Figure2: Gram staining

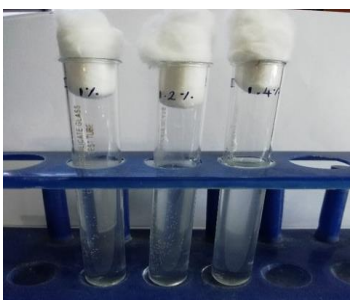

Figure 3: Ethanol tolerance test

Figure 4: Triple sugar iron test

Figure 5: Carbohydrate Fermentation test

Figure 6: Pure culture of *Saccharomyces cerevisiae* in Yeast Extract Peptone Broth

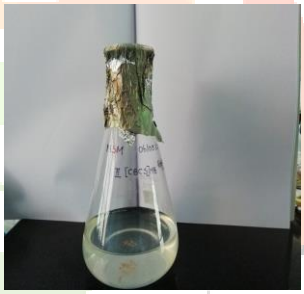

Figure 7:Mineral Salt Medium

Figure 8: Inoculate the crude oil and isolate into sample flask control flask without sample contain only crude oil

Figure 9: After 28 days, the crude oil biodegradation *Saccharomyces cerevisiae*

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