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Utilization of banana peel as a substrate for ethanol production by isolated yeast strains

• **Menka Mrinal** • **Sonal Sinha**
• **Sonal Suman**

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Corresponding Author : Sonal Suman

Abstract : *In the present study production of bioethanol was investigated using yeast strains isolated from ripe bananas and comparative assessment of their potential for bioetanol production was studied. Out of the seven isolates, five showed enhanced ability and were subsequently identified and assessed for ethanol production in which Strain I showed no ethanol production, Strain II and III showed less ethanol production and Strain IV and V showed more ethanol production. The obtained results shows production of ethanol from ripe banana peels was quite significant when compared with its sugar content. This is a cost effective method which may helps in waste management and help in overcoming the energy crisis in the world.*

Key Words: *Banana peels, Yeast, Fermentation, Bioethanol*

Menka Mrinal
B.Sc. III year, Industrial Microbiology (Hons.),
Session : 2011-2014, Patna Women's College,
Patna University, Patna, Bihar, India

Sonal Sinha
B.Sc. III year, Industrial Microbiology (Hons.),
Session : 2011-2014, Patna Women's College,
Patna University, Patna, Bihar, India

Sonal Suman
Assistant Professor, Deptt. of Industrial Microbiology,
Patna Women's College, Bailey Road,
Patna – 800 001, Bihar, India.
E-mail : sonal.micro89@gmail.com

ntroduction :

Ethanol is a relatively low cost alternative fuel. Ethanol is the only clean burning liquid fuel available that can replace oil. Ethanol production is usually accomplished by chemical synthesis of petrochemical substrate and microbial conversion of carbohydrates present in agricultural products (Goettemoeller and Goettemoeller, 2007).

The preferred strain for industrial production of ethanol has been *Saccharomyces cerevisiae* (Ameh et al. 1989). Certain fermentation parameters such as inoculum, enzyme, and substrate concentration besides optimum pH, temperature and time play an important role in obtaining good ethanol yield. Bioethanol, unlike petroleum, is a form of renewable source of energy that can be produced from various sources, like household waste, agricultural waste, fruit juices, fruit wastes etc.

Banana is one of the major food resources in the world. These cultures occupy the 4th world rank of the most significant food stuffs after rice, corn and milk (*FAO* 1999*; INI BAP* 2002) As per FAO statistics, India is the largest producer of banana

in the world and accounts for nearly 30% of the total world production of banana. Though banana peel is a fruit residue, it accounts for 30-40% of the total fruit weight (Emaga et al. 2008) and contains carbohydrate, proteins, and fibre in significant amount. Since banana peels contain lignin in low quantities (Hammond et al. 1996) it could serve as a good substrate for production of value added products like ethanol.

Banana peels are readily available agricultural waste, yet they seem to be underutilised as potential growth medium for local yeast strains, despite their rich carbohydrate content and other basic nutrient that can support yeast growth (Brooks 2008). Protein is essential nutrient for yeast growth while fat is vital to the structure and biological functions of the cell and can be utilised alternative source of energy by the cells (Dhabekar and Chandak 2010). The aim of this study therefore is to use yeast strain and ripe banana peels as a substrate for ethanol production. This source is always available in abundance and thus serves as readily available raw material for the production of ethanol, it is cost effective, it can also help in waste management and contribute to reduce pollution in environment.

Materials and Methods :

Source : The ripe banana peels used in this study were collected from kitchen scrap and local fruit market of Patna. The fresh ripe peels were used within 24 hours after collection.

Isolation of Yeast : Glass wares such as beakers, petri plates, test tubes and conical flasks were thoroughly washed, sterilized and kept in a hot air oven for further use.

The ripe banana pulp and peels were mashed aseptically. Serial dilution of the mash was done up to 10^{-4} dilution and inoculated on Yeast Extract Peptone Agar media (Brooks 2008) and incubated at 26°C temperature for 24-48 hours. The isolates were purified by repeated subculturing after every 1 week.

Identification of Yeast Strains and Biochemical analysis : Specifically, the identification parameters included colonial and morphological characteristics. The colonies isolated from the banana pulp and peel on Yeast Extract Peptone Agar (YEPA) media plates were characterized by Lactophenol - Cotton Blue staining (3:1) and standard biochemical analysis. After the identification of yeast strains they were analysed on biochemical basis by their capability to be resistant to antibiotic (chloramphenicol) and ability to utilise urea (Hupert et. al. 1975).

Screening of the isolated Yeast for fermentation ability : The isolates were screened for fermentation ability by performing fermentation test. Phenol red sugar broth (10g peptone, 5g NaCl, 5g sugar, 0.018g phenol red, 1000 ml distilled water) was prepared for two different sugars (glucose and fructose) and inoculated with isolated Yeast strains. It was then incubated at 26^0C for 4 days and thereafter the strains were selected based on the volume of the gas in the durham tube during the incubation period (Brooks 2008).

After determining the fermentation ability of the yeast strains, the indigenous yeast with good fermentation attributes which may enhance the ethanol production is used for ethanol production.

Preparation of agro waste broth : Modified Banana Peel (MBP) broth was prepared using the method of Essien et al. (2005). Fresh banana peels were chopped into small pieces and dried in hot air oven at 70^0C for 2 days and then milled in electric

blender to produce banana peel powder. About 300g of the powder was washed in sterile distilled water and filtered through two-layered cheese cloth to obtain primary extract. The primary extract was then filtered through Whatman filter paper 1 to obtain the experimental filterate, which was used for the preparation of Modified Banana Peel broth for the isolation of local yeast strains.

The broth was modified with the addition of malt extract (12g for 600ml filtrate) and sterilized by autoclaving at 121^{0}C for 30 min. The pH of the broth was adjusted to 4.5 to discourage the growth of bacteria.

Ethanol production : 100ml of MBP broth was poured in six 250ml of uninoculated flask and each flask was inoculated with selected isolated yeast strain. One was kept as comparative control. It was then incubated for one week at 26°C (Brooks 2008). During the incubation period qualitative analysis of the filterate was done to confirm the production of ethanol. The qualitative tests done were smell test of ethanol, Phenol test, Esterification test and Iodoform test.

Reducing Sugar test of culture broth : The total reducing sugar content of the broth was determined by Dinitrosalicyclic (DNS) method (Miller 1959) with glucose as standard.

Results and Discussion :

Total yeast isolated : Seven different colonies at different dilutions were isolated on YEPA media plates having characteristic colour, texture, margin, shape and elevation (Table 1).

Single colonies were isolated from the dilutions (10^{-2}, 10^{-3}, 10^{-4}) and two different colonies were isolated from the plates directly inoculated with banana pulp and banana peel (Fig. 1 and 2).

Table 1. Cultural Characteristics of the isolated yeast

Characteristics	10^{-2} dilution	10^{-3} dilution	10^{-4} dilution	Banana Pulp		Banana Peel	
				Colony 1	Colony 2	Colony 1	Colony 2
Colour	Creamy	Creamy	Creamy	White	Creamy	Creamy	White
Texture	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy	Slimy
Shape	Irregular	Circular	Circular	Circular	Circular	Irregular	Irregular
Margin	Irregular	Smooth	Smooth	Smooth	Irregular	Irregular	Smooth
Elevation	Flat	Flat	Flat	Flat	Flat	Flat	Flat

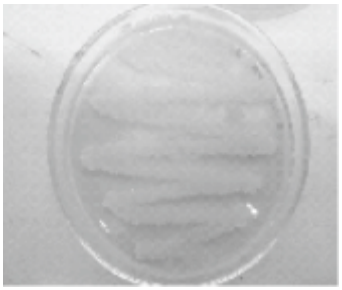

Fig 1. Yeast colony on YEPA media

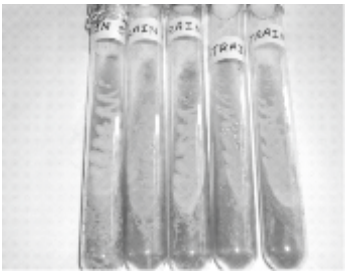

Fig 2. Yeast colonies on YEPA slants

Microscopic characteristics of isolated strains : Isolated yeast colonies stained with lactophenol and cotton blue was viewed under the microscope and the observations were recorded in Table 2.

Table 2. Microscopic characteristics of isolated strains

Strains	Characteristics			
	Surface	Margin	Cells	Budding
Strain I	Smooth	Circular	Spherical	Multipolar
Strain II	Smooth	Circular	Spherical	Unipolar
Strain III	Smooth	Circular	Oval	Bipolar
Strain IV	Smooth	Circular	Oval	Unipolar
Strain V	Smooth	Circular	Spherical	Bipolar
Strain VI	Rough	Irregular	Ellipsoidal	Multipolar
Strain VII	Rough	Irregular	Ellipsoidal	Bipolar

Biochemical analysis of the isolated Yeast strains

(a) Resistant to antibiotic : Growth was observed in all the plates, which shows all the strains were resistant to the antibiotic chloramphenicol.

(b) Nitrate Utilization test : Strain I, II, III, IV, V gave positive result by the formation of blue colour which shows that these strains were utilizing nitrogen whereas Strain VI and VII gave less intense blue colour indicating that they utilize less amount of nitrogen.

Screening of the isolated yeast strains : The isolates were subjected to fermentation test for glucose and fructose to check their fermentation ability, where the strains showed a positive result for acid and gas formation and change in colour from red to yellow was observed along with bubble formation (Table 3).

Table 3. Observation of fermentation test

Strains	48 hours				72 hours			
	Glucose		Fructose		Glucose		Fructose	
	Acid formation	Gas formation	Acid formation	Gas formation	Acid formation	Gas formation	Acid formation	Gas formation
I	+	-	+	+	+	+	+	+
II	+	+	+	+	+	+	+	+
III	+	-	+	+	+	+	+	+
IV	+	+	+	+	+	+	+	+
V	+	+	+	+	+	+	+	+
VI	+	-	+	-	+	-	+	-
VII	+	-	+	-	+	-	+	-

\+ shows "positive result"
\- shows "negative result"

Out of seven isolates, five Strains (Strain I, II, III, IV & V) possessed best fermentation ability as they showed positive result for acid formation and gas production. Hence, five strains were selected on the basis of fermentation test and gas produced for further studies.

Estimation of Reducing Sugar : Amount of sugar reduced in MBP broth was tested by DNSA method.

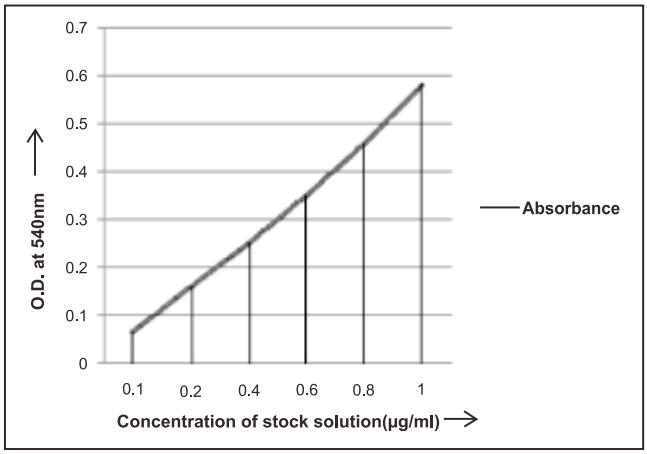

Fig 3. Standard Graph of reducing sugar

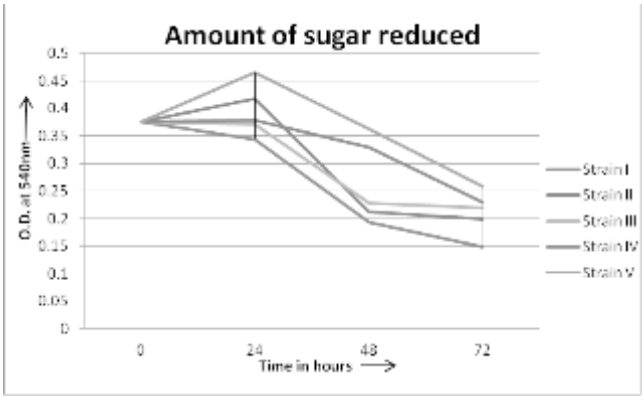

Fig 4. Graph showing amount of sugar reduced

Thus, estimation of reducing sugar showed that with increasing time the amount of sugar gets reduced which denotes the increase in alcohol production (Fig. 3 and 4).

Test for Bioethanol production (Qualitative analysis)

(a) Phenol test

Table 4. Observation of Phenol test

Time (hours)	StrainI	StrainII	StrainIII	StrainIV	StrainV
24	-	-	-	-	-
48	-	+	+	+	+
72	-	+	+	++	++

\+ shows "positive result"
– shows "negative result"

Strain I showed negative result. Strain II, III, IV, V showed positive result in which Strain IV and Strain V showed more colour change (Table 4).

(b) Esterification test

Table 5. Observation of Ester test

Time (hours)	StrainI	StrainII	StrainIII	StrainIV	StrainV
24	-	-	-	-	-
48	-	+	+	+	+

\+ shows "positive result"
– shows "negative result"

Strain I showed negative result due absence of fruity smell. Strain II and III gave a specific fruity smell whereas Strain IV and V shows more presence of fruity smell (Table 5).

(c) Iodoform test

Table 6. Observation of Iodoform test

Time (hours)	StrainI	StrainII	StrainIII	StrainIV	StrainV
24	-	-	-	-	-
48	-	+	+	+	+
72	-	+	+	++	++

\+ shows "positive result"
– shows "negative result"

Strain I showed negative result due to absence of yellow crystals. Strain II and III showed little formation of yellow colour whereas Strain IV and V gave more positive result with the formation of more yellow crystals (Table 6).

Hence, from the qualitative analysis of all the five Strains it can be concluded that Strain I showed negative result in ethanol production. Strain II and III showed a slight positive result whereas Strain IV and V showed more positive result showing more production of ethanol.

The result of this study indicated that indigenous yeasts with good fermentation attributes, which may enhance ethanol yield and minimize cost of production, could be obtained from ripe banana peels. Banana peels are always available in abundance in India and thus serve as readily available raw materials for the isolation of ethanol yeasts. The import of this study is that it has been able to produce five yeast strains with appreciable fermentation ability. Although the level of ethanol tolerance recorded in this study is less then that reported by Benitez et al. (1983), the isolates could be manipulated genetically for higher ethanol tolerance. The proximate analysis of ripe banana peels was not determined in this study, but Essien et al. (2005) recorded crude protein and crude fat contents of 7.8 and 11.6%, respectively in banana peels.

There is no as such use of banana peels and hence treated as a waste. Banana peels are always available in abundance in India and thus serve as readily available raw substrate for the production of ethanol. This is a cost effective way and it also helps somewhat with the energy crises in this fast moving World. Thus, alternative sources like banana wastes could be used for bioethanol production.

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