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Full Length Research Paper

Ethanol production potential of local yeast strains isolated from ripe banana peels

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The ability of different yeast strains isolated from ripe banana peels to produce ethanol was investigated. Of the 8 isolates screened for their fermentation ability, 5 showed enhanced performance and were subsequently identified and assessed for important ethanol fermentation attributes such as ethanol producing ability, ethanol tolerance, flocculence, thermo-and osmo-tolerance. *Saccharomyces cerevisiae* R-8 exhibited the best attributes for ethanol production by being highly flocculent, tolerant to 6 - 12% ($^{v}/_{v}$) ethanol, fermentatively active at 37 - 42^{o}C and fermented 40% ($^{v}/_{v}$) glucose. *S. cerevisiae* T-7 and *S.cerevisiae* R-2 showed rapid fermentative activity on maltose by liberating 150 and 120 µl of CO_2 in 6 h, respectively. *Debaryomyces hansenii* B-2 and *Saccharomyces kluvveri* K-6 each fermented 40% (($^{v}/_{v}$) glucose at 30^{o}C to yield 3.6 and 5.8% ethanol, respectively. The five yeast strains are therefore potential candidates for ethanol production from banana peels or other local starch sources.

Key words: Banana peels, ethanol, yeast, fermentation, starch.

INTRODUCTION

The increasing demand for ethanol for various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives, has necessitated increased production of this alcohol. Ethanol production is usually accomplished by chemical synthesis of petrochemical substrates and microbial conversion of carbohydrates present in agricultural products. Owing to the depleting reserves and competing industrial needs of petrochemical feed stocks, there is global emphasis in ethanol production by fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology. According to Stewart et al. (1984), an ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, appreciable osmotolerance, enhanced ethanol tolerance and good thermotolerance. Although no microbial strain has all these desirable qualities, few yeast strains have been found to possess appreciable characteristics for ethanol production (Panchal et al., 1982 and Hacking et al., 1984). The technological behavior of industrial microorganisms remains the main stay of industrial secret in fermentation industry, hence most industrial microorganisms are patented and may not be available for use outside their country of origin. This is of serious economic concern as it does not allow for rapid expansion of fermentation industries, hence the need to source for indigenous and suitable yeast strains from local substrates for sustainable ethanol production.

Various strains of indigenous yeasts capable of producing ethanol have been isolated from different local sources such as molasses (Rose, 1976), sugar mill effluents (Anderson et al., 1986) and local fermented foods (Ameh et al., 1989) and fermented pineapple juice (Eghafona et al., 1999). In most of these studies, the preferred candidate for industrial production of ethanol has been *Saccharomyces cerevisiae.* This yeast also has the ability to produce ethanol which is not contaminated by other products from the substrate.

Banana peels are readily available agricultural waste in Nigeria, yet they seem to be underutilized as potential growth medium for local yeast strains, despite their rich carbohydrate content and other basic nutrients that can support yeast growth. The aim of this study therefore is to isolate indigenous yeast strains from ripe banana peels

and evaluate their potential for ethanol production.

MATERIALS AND METHODS

Source of banana peels

The ripe banana peels used in this study were of the variety *Musa x paridasiaca* fruit, obtained from local markets in Akwa Ibom State, Nigeria. The fresh ripe peels were used within 24 h after collection. When the peels could not be used immediately they were stored in the refrigerator to prevent deterioration. However, the stored samples were utilized within 48 h of collection.

Processing

The ripe peels were chopped into small pieces using clean knife which was disinfected with 70% ethanol. The chopped pieces were sun dried under mild sunlight and then milled in electric blender to produce banana peel mash. About 300 g of the mash was washed in sterile distilled water and filtered through two-layer cheese cloth to obtain the primary extract. The primary extract was then filtered through Whatman No.1 filter paper to obtain the experimental filtrate, which was used for the preparation of modified banana peel agar for the isolation of local yeast strains.

Preparation of modified banana peel agar (MBPA)

The MBPA was prepared using the method of Essien et al. (2005) with some modification. This involved dissolving 10.0 g of malt extract agar in 500 ml of the experimental filtrate and incorporating 0.75% ($^{W}/_{V}$) mycological peptone (Oxoid) and 0.5% ($^{W}/_{V}$) sodium propionate. The medium was sterilized by autoclaving at 121°C for 15 min. Thereafter, the pH of the medium was adjusted to 4.5 with 0.5 M HCl. The acid pH was to discourage the growth of bacteria while the use of sodium propionate discouraged the growth of moulds.

Isolation of yeast strains

The yeast strains were isolated using the method of Ameh et al. (1989), on serially diluted samples of the experimental filtrate. The last two dilutions were plated in duplicates using pour plate technique described by Madigan et al. (1997). All the plates were incubated at 30°C for 24-48 h. The isolates were purified by repeated subculturing and preserved on slants of the same medium (MBPA) at 4°C .

Characterization and identification of yeast strains

These were done using the procedures described by Barnett et al. (1983). Specifically, the identification parameters included colonial and morphological characteristics, and assimilation of some carbon compounds.

Assessment of the yeasts for attributes essential for ethanol production screening

Each isolate was screened for fermentative ability by inoculating 2.0 ml of the yeast suspension into 8.0 ml of fermentation medium in a test tube carrying inverted Durham tube. The medium contained ($^{W}/_{V}$) glucose 2.0%, yeast extract (Oxoid) 0.3% and peptone (Oxoid) 0.5%. The tubes were incubated at the room temperature for 48 h and thereafter the strains were selected based on the volume of gas in the Durham tube during the incubation period.

Fermentation of 40% ($^{W}/_{V}$) glucose

This test was carried out using the banana peel-yeast extract peptone fermentation medium earlier described, except that the concentration of glucose was increased to 40% ($^{W}/_{V}$) and the incubation period was 6 h.

Flocculating ability

The flocculating ability of the selected yeast strains was tested using the method described by Ameh et al. (1989) with some modifications. This involved growing the test stains separately for 48 h in banana peel broth supplemented with yeast extract and mycological peptone. The yeasts were harvested by centrifugation at 4500 x g for 30 min and washed twice with sterile distilled water. The washed cells were dried at 45°C to constant dry weight, and 5.0 g of each strain was suspended in 100 ml of distilled water and mixed with 1.0 ml of acetate buffer (pH 5.0) in graduated centrifuge tubes and allowed to stand for 10 min.

The amount of sediment formed after 10 min, indicated whether or not the selected strains were flocculent if the amount of sediment formed ranged from 0.5 – 1.0 ml, and as non-flocculent if the amount of sediment was less than this range.

Fermentation of glucose at 37 and 42°C

This test was conducted, using the semi-synthetic medium described earlier in this paper to assess the thermotolerance of the selected yeast strains. The incubation was at 37and 42°C for 48 h.

Fermentative potential

This was determined quantitatively by measuring the amount of carbondioxide evolved during the fermentation of 2 and 40% ($^{W}/_{V}$) glucose for 6 h by means of Warburg manometry. The medium used was the semi-synthetic banana peel-yeast extract-peptone broth, while incubation was at 28°C.

Ethanol production

The ability of the selected strains to produce ethanol was determined by inoculating 5.0 ml of the yeast suspension separately into two 500 ml Erlenmeyer flasks containing 100 ml of the fermentation medium supplemented with 10% ($^{W}/_{V}$) glucose. One flask was incubated at 30°C while the other was at 37°C for 48 h, respectively.

The percentage ethanol by volume was determined from the table correlating percentage volume of ethanol with specific gravity at 20°C according to the methods of A.O.A.C (1990).

RESULTS

A total of 5 yeast strains were selected based on their ability to ferment glucose and identified to species level. These were *S. cerevisiae* (3 strains), *D. hansenii* (1 strain)

Table 1. Attributes of the yeast strains isolated from waste banana peels for ethanol production.

Yeast strain	Ethanol yield in 10% ($^{V}/_{V}$) glucose		Flocculation rate (ml/10min)	Fermentative capacity (µlCO_2) with 2% glucose		Fermentative capacity (µlCO_2) with 40% glucose at 37°C	Ethanol tolerance			
	30°C	37°C		37°C	42°C		6%	8%	10%	12%
S. cerevisiae R-2	4.6[a]	3.0	0.80	170	120	140	+	+	-	-
S. cerevisiae R-8	7.2	4.8	2.6	320	150	190	+	+	+	+
S. cerevisiae T-7	4.3	4.0	1.00	160	100	125	+	+	-	-
S. *kluyveri* K-6	4.8	3.8	0.40	80	46	54	+	+	-	-
D. *hansenii* B-2	3.6	3.1	0.20	40	-	-	+	-	-	-

[a]The values are means from three determinations
+ = Tolerant ; - = Not tolerant.
Fermentation period was 6 h.

Table 2. Fermentation of simple sugars other than glucose by the yeast strains isolated from waste banana peels.

Yeast strain	Rate of fermentation of the sugars (µl CO_2/ 6 h)							
	Cellobiose	Maltose	Mannose	Galactose	Ribose	Xylose	Sucrose	Arabinose
S. cerevisiae R-2	-	150.0	84.60	42.60	-	-	60.40	-
S. cerevisiae R-8	-	100.0	120.80	39.20	-	-	130.0	-
S. cerevisiae T-7	-	76.0	64.10	36.40	-	-	34.60	-
S. *kluyveri* K-6	-	47.0	70.40	20.50	-	-	40.0	-
D. *hansenii* B-2	-	53.0	41.30	30.40	-	-	30.40	-

The final concentration of all the sugar used was 2% ($^{V}/_{V}$).
Values are means from two determinations.

and *S. kluyveri* (1 strain). The result of the assessment of the 5 yeast stains for attributes essential in industrial fermentation of ethanol is presented in Table 1. Only *S. cerevisiae* R-8 was highly flocculent. The rest were poorly flocculent. There were variations in the rate of tolerance to different levels of alcohol. *D. hansenii* B-2 and *S. klyveri* K-6 tolerated 6 and 8% ethanol, respectively, while *S. cerevisiae* R-8 was tolerant to 12% ($^{V}/_{V}$) ethanol. This table also shows the thermotolerance exhibited by the various yeast strains and their ability to ferment 40% glucose. *S. cerevisiae* R-8 rapidly fermented 2% ($^{W}/_{V}$) glucose at 37 and 42°C with the liberation of 320 and 150 µl of CO_2, respectively. *S. cerevisiae* R-2 and *S. cerevisiae* T-7 exhibited moderately rapid fermentation, while *D. hansenii* and *S. kluyveri* exhibited low rates of fermentation at both temperatures Table 1 also shows the ethanol production capability of the five strains of yeasts at 30 and 37°C respectively. The result shows that all the strains performed better at 30°C. *S. cerevisiae* R-8 was the most active strain at both temperatures, yielding 7.2% (V/V) and 4.8% (V/V) ethanol at 30 and 37°C respectively. At 37°C the yields of ethanol produced by *D. hansenii, S. kluyveri* and *S. cerevisiae* T-7 were 3.1, 3.8 and 4.0% (V/V) respectively. Except *D. hansenii*, other strains fermented 40% glucose rapidly at 37°C, with *S. cerevisiae* R-8 being the best, having produced the highest level of CO_2 (190µl of CO_2) at 37°C in 6h.

Table 2 shows the fermentation of simple sugars other than glucose by five strains of yeast isolated from waste banana peels. *S. kluyveri* and *D. hansenii* showed minimal fermentation rates on galactose, liberating 20.50 and 30.40µ1 of CO_2 in 6h respectively. All the five strains failed to ferment arabinose, ribose, cellobiose and xylose.

Table 3 shows the percentage viability of the different yeast strains at 12% concentration of ethanol. *S. cerevisiae* R-8 shows the highest tolerance with 70% viability while *D. hansenii* showed the least tolerant with 30% viability at this concentration.

DISCUSSION

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 Southern Nigeria and thus serve as readily available raw materials for the isolation of ethanol yeasts. In this study, *S. cerevisiae* R-8 exhibited considerable potential for industrial production

Table 3. Percentage viability of the yeast strains isolated from waste banana peels in 12% ($^{v}/_{v}$) ethanol incubated at 30°C.

Yeast strain	Mean viable counts (x10^4 cfu/ml)			Cell viability (%)
	0 h	24 h	48 h	
S. cerevisiae R-2	2.8 ± 0.03	4.3 ± 0.08	1.32 ± 0.01	47.0
S. cerevisiae R-8	4.2 ± 0.14	6.4 ± 0.11	2.94 ± 0.18	70.0
S. cerevisiae T-7	2.3 ± 0.76	4.0 ± 0.24	1.22 ± 0.03	53.0
S. *kluyveri* K-6	3.6 ± 0.01	5.0 ± 0.03	1.44 ± 0.11	40.0
D. *hansenii* B-2	3.0 ± 0.04	4.4 ± 0.14	0.60 ± 0.06	30.0

Cell viability (%) = (Viable count at 48 h / Viable count at 0 h) x 100
Values are means ± standard deviation from three determinations.

of ethanol. The strain exhibited high flocculation ability, good osmo-and thermo-tolerance and fermented higher concentrations of sugar to yield appreciable amount of ethanol. These attributes are the requisite criteria for selecting yeasts as candidates for industrial production of ethanol (Hacking et al., 1984; Rose, 1976). However, this strain could not ferment pentose sugars and cellobiose and may therefore not be suitable for the fermentation of cellulosic materials. Benitez et al. (1983) described wine yeasts which could grow well at 10% ($^{V}/_{V}$) and fairly well at 15% ($^{V}/_{V}$) ethanol. 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. Protein is essential nutrient for yeast growth while fat is vital to the structure and biological functions of the cells and can be utilized as alternative source of energy by the cells. It appears therefore that the impressive performance of yeasts in banana peels is due, partly, to the high contents of fat and protein.

The import of this study is that it has been able to produce 5 yeast strains with appreciable fermentation ability. Although the ethanol yield is low, maximum being 7.2% produced by *S. cerevisiae* R-8, the strains could be genetically manipulated under suitable environment for higher yield of ethanol .

REFERENCES

Ameh JB, Okagbue RN, Ahman AA (1989). Isolation and characterization of local yeast strains for ethanol production. Niger. J. Technol. Res. (1): 47-52.

Anderson PJ, McNeil K, Watson K (1986). High efficiency carbohydrate fermentation to ethanol at temperatures above 40°C by *Kluyveromyces marxianus Var. maxianus* isolated from sugar mill. Appl. Environ. Microbiol. 51(6): 1124-1129.

AOAC (1990). Official method of analysis. Association of Official Analytical Chemist, 15th Ed., U.S.A.

Barnett JA, Payne RW, Yarrow D (1983). Yeasts: Characteristics and Identification. Cambridge University Press. Cambridge, pp. 19-28.

Benitez T, Del Casttillo L, Aguilera A, Conde J, Oimedo EC (1983). Selection of wine yeasts for growth and fermentation in the presence of ethanol and sucrose. Appl. Environ. Microbiol. (45)5: 1429-1436

Eghafona NO, Aluyi HAS, Uduehi IS (1999). Alcohol yield from pineapple juice: Comparative study of *Zymomonas mobilis* and *Saccharomyces uvarum.* Niger. J. Microbiol. 13: 117-122.

Essien JP, Akpan EJ, Essien EP (2005). Studies on mould growth and biomass production using waste banana peels. Bioresour. Technol. 96: 1451-1456.

Hacking AJ, Taylor LWF, Hamas CM (1984). Selection of yeast able to produce ethanol from glucose at 40°C. Appl. Microbiol. Biotechnol. 19: 361-363.

Madigan MY, Martinko JM, Parker J (1997). Brock's Biology of Microorganisms (8th edn.) U.S.A; Prentice Hall International Incorporated.

Panchal CJ, Peacock I, Stewart GG (1982). Increased osmotolerance of genetically modified ethanol producing strains of *Saccharromyces* sp. Biotechnol. Lett. 4(10): 639-644.

Rose D (1976). Yeasts for molasses alcohol, Proc. Biochem. 11(2): 10-12, 36.