African Journal of Biotechnology Vol. 4 (7), pp. 660-662, July 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Short Communication

Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice

Osho, A.

Department of Biological Sciences, Olabisi Onabanjo University, Ago-Iwoye, Nigeria. E-mail: osho_adeleke@yahoo.com.

Accepted 11 April, 2005

Seventeen wine yeasts isolated from fermenting cashew apple juice were screened for ethanol and sugar tolerance. Two species of *Saccharomyces* comprising of three strains of *S. cerevisiae* and one *S. uvarum* showed measurable growth in medium containing 9% (v/v) ethanol. They were equally sugar-tolerant having good growth in medium containing 25% (w/v) glucose. Two of the strains (*S. cerevisiae*) were found to posses higher invertase activities than the remaining two. Further search for industrially useful yeasts in tropical fruits is suggested.

Key words: Ethanol tolerance, Invertase activities, wine yeast, cashew, cashew apple juice.

INTRODUCTION

In assessing a yeast strain for industrial use, specific physiological properties are required (Ekunsanmi and Odunfa, 1990). Ethanol tolerance, sugar tolerance and invertase activities are some of the important properties for use in industrial ethanol production (Jameonoz and Benitez, 1986).

Yeasts have been isolated from many sources for industrial purposes. Such include yeasts isolated from palm wine for industrial production of ethanol (Layokun, 1984), for single cell protein (Amachukwu et al., 1986), for leaving of dough for bread-making (Oakagbue, 1988) and for wine production (Osho and Odunfa, 1999).

Yeasts have also been isolated from many fermenting sources including fermenting cassava tubers (Okafors, 1977; Oyewole and Odunfa, 1988). Although, the use of cashew apple juice as a substrate for single cell protein has been reported (Layokun et al., 1986; Osho, 1995). No work has been done in assessing the yeasts isolated from cashew juice for any characteristics of industrial importance.

The work reported here was directed at assessing yeasts from fermenting cashew apple juice for ethanol tolerance, sugar tolerance and invertase activities, which are some of the properties required of yeasts to be utilized for industrial ethanol and wine production.

MATERIALS AND METHODS

Isolation of yeasts

The fruits were washed and rinsed many times in distilled water. They were then cut, squeezed and the juice collected in separate sterile flasks. Samples of the juice were diluted serially and 0.1 ml of diluted and undiluted samples were plated on yeast extract peptone-dextrose agar medium (YEPD) supplemented with 0.1 mg/ml streptomycin sulphate as previously described (Osho, 1995). The plates were incubated at 30°C for 24 to 48 h. Morphologically distinguished colonies were then selected under a dissection microscope. The yeasts were purified by subsequent streaking on YEPD medium. Pure culture of each strain was kept on YEPD agar slants and stored at 40°C until needed.

Screening of Yeast for ethanol tolerance

The medium of Novak et al. (1981) was used for the screening of the yeast for ethanol tolerance. The medium was sterilized at 121°C for 15 mm in an autoclave and cooled. Enough absolute ethanol was then added to different flask of the same medium to constitute varying percentages of ethanol differing by 1% (v/v) from one flask to the other. 40 ml portion of the media were distributed into 100 ml conical flask respectively. The media were duplicated and inoculated separately with each of the yeast strain. The initial optical density of each flask was read off on a Pye-Unicam SP6 spectrophotometer at 615 nm against the medium as blank. The inoculated flasks were transferred in a gyrotary shaker incubator set at 150 ppm at 30°C for 72 h. The increase in optical density in a flask was recorded as evidence of growth. The concentration of alcohol at which the growth of the yeast was just inhibited was assessed as the ethanol tolerance of the yeast. Only the yeast strains that showed growth in 9% ethanol (v/v) were further examined.

Sugar tolerance of ethanol-tolerance yeasts

The procedure by Ekunsanmi and Odunfa (1990) was employed. The medium was sterilized by autoclaving at 121°C for 15 min, cooled and inoculated with 0.1 ml of cell suspension of each

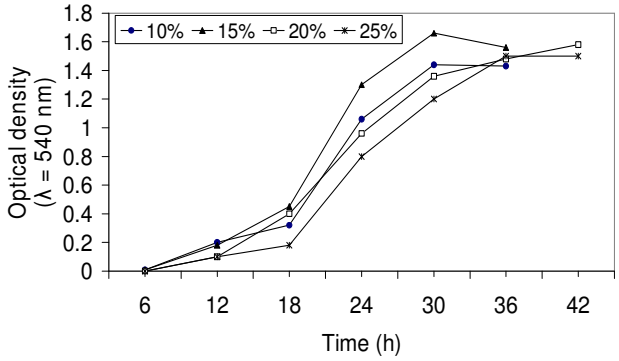

Figure 1. Growth curve of BSOSU 0271 in increasing sugar concentrations (%, w/v).

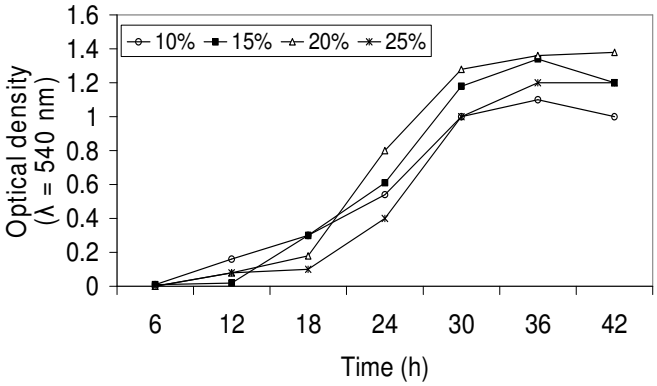

Figure 2. Growth curve of BSOSU 0269 in increasing sugar concentrations (%, w/v).

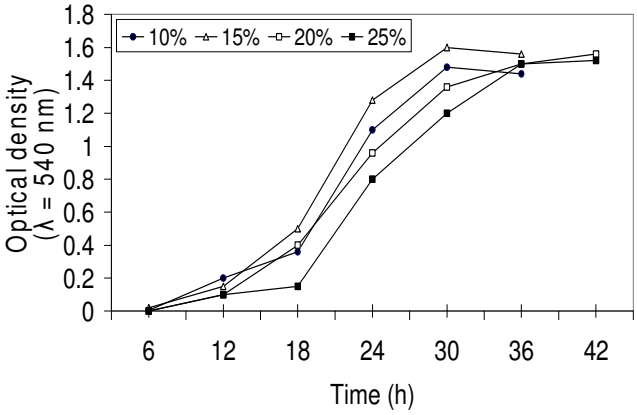

Figure 3. Growth curve of BSOSU 0260 in increasing sugar concentrations (%, w/v).

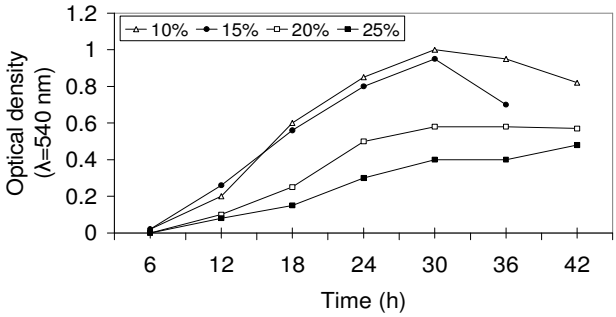

Figure 4. Growth curve of BSOSU 0275 in increasing sugar concentrations (%, w/v)

isolated strain. The turbidity was measured by spectrophotometer at 540 nm after inoculation and subsequently at intervals of 12 h for 60 h. The medium used contained yeast extract, 10 g/L; peptone, 20 g/L, and glucose added in concentrations of 100, 150, 200 and 250 g/L.

Determination of invertase activities of yeasts

Yeast strains grown on the agar slants were harvested by pouring sterile distilled water into the slants and gently scraping with a wire loop. The cells were washed, centrifuged and 0.1 g wet weight of each was re-suspended in 10 ml of acetate buffer, pH 5.0 (Jimenez and Benitez, 1986), sucrose solution (4% w/v, 2 ml) in the same acetate buffer was inoculated with 1 ml of cell suspension for 5 min at 30°C. The amount of reducing sugar released was determined by dinitro-salicylic acid method (Bernfield, 1951). The amount of enzyme which liberate 1 µmole reducing sugar per minute was defined as one unit of invertase activity.

Characterization and identification of cultures

The yeast isolates were characterized by the conventional methods as described by Kreger van Rij (1984). Further identification of the isolates was done in accordance with the proposed scheme of Deak and Beuchat (1987). The carbon assimilation tests were made with API (ATB 32 C) (API System, Montalieu Vercieu, France). The isolates were deposited in the industrial yeast collection of the Department of Biological Sciences, Ogun State University, Ago-Iwoye.

RESULTS

The seventeen morphologically different yeast strains (BSOSU 0260 – 0277) were obtained from the fermenting cashew apple juice. Four of the seventeen isolates were able to grow in 10% (v/v) ethanol concentration and above. Table 1 shows the identities of the isolates, the minimum percentage of ethanol (v/v) which inhibited their growth and the invertase activities of the isolates. BSOSU 0273 showed less tolerance to ethanol than the other species of the *S. cerevisiae.* All the four strains of the *Saccharomyces* species were able to grow in all the sugar concentrations (Figures 1 - 4). Except for *S. cerevisiae* (BSOSU 0269) which recorded its highest growth rates in 20% (w/v), all the yeast strains had their

maximum growth rates in the sugar concentrations of 15% (w/v). Also, growth rate remain lowest in 25% (w/v) of sugar concentrations in all the yeast strains. The highest growth rate was shown by the strain BSOSU 0271. Only slight differences were observed in the growth rates with increasing sugar concentrations, the differences being most obvious between 10 and 25% sugar concentrations (Figures 1 and 2). The effect of increased glucose concentration on BSOSU 0260 was similar to that of BSOSU 0275. Increasing sugar concentrations for these strains prolonged their lag phases for 12 h in the media containing 20 and 25% glucose respectively. Also reduced growth rates were equally observed in these media. The invertase activity of BSOSU 0271 was the highest and this was followed by BSOSU 0269. The least activity was found in BSOSU 0275 (Table 1).

Table 1. Ethanol tolerance levels and invertase activities of selected yeasts from fermenting cashew apple juice.

Species with BSOSU* accession no.	Ethanol tolerance (%, v/v)	Invertase activity (µmol/min)
S. cerevisiae 0271	12	38.9
S. cerevisiae 0269	11	19.7
S. cerevisiae 0260	10	8.8
S. uvarum 0275	9	8.1

*BSOSU = Industrial yeast collection of the Department of Biological Sciences, Ogun State University, Ago-Iwoye.

DISCUSSION

The ability of the 4 ethanol-tolerant yeast strains to withstand osmotic stress has been amply demonstrated by the fact that the yeast strains were able to grow in media containing relatively high degree of sugar concentrations. This observation is in agreement with the suggestion of Gray (1945) who stated that ethanol-tolerant yeasts tend to be sugar-tolerant. Ekunsanmi and Odunfa (1990) asserted that the combination of sugar tolerance and alcohol tolerance is an advantage when a yeast is being considered for industrial use especially where ethanol is being produced. Jimenez and Benitez (1986) and Du Preez et al. (1987) pointed out that ethanol tolerance is particularly important since ethanol tolerance can hardly be avoided during fermentation although substrate inhibition can be avoided through stepwise addition of substrate. In medium where wine is the ultimate product, sugar tolerance by the wine yeast strains will allow larger initial amount of sugar to be used.

Harrison and Graham (1970) have stated that high invertase activity is required in yeasts for growth in medium in which the principal carbohydrate is sucrose.

Thus, the yeast strains BSOSU 0271 and BSOSU 0269 should be suitable for the production of wines where the desired alcohol levels is between 11 and 12%; while BSOSU 0260 and BSOSU 0275 could be used where the desired level is between 9 and 10%. Cashew apple has been reported to be good for wine production and which commercial production has started in India (Osho and Odunfa, 1999). The isolation of high ethanol-tolerant and sugar-tolerant yeast strains from cashew has revealed the need to look further into other tropical fruits which might yield new strains of wine yeasts.

REFERENCES

Amanchukwu SC, Okpokwasili GC, Obafemi A (1986). Evaluation of *Schzossacharomyces pombe* isolated from palm wine for single cell protein using hydrocarbon feedstock. In: Abstracts 14th Annual Conference, Nigerian Society for Microbiology pp. 7-8. University of Calabar, Calabar, Nigeria.

Amerine MA, Berg HW, Ough CS, Kunkee RE, Singleton NL, Webb AA (1980). The technology of wine making. Westport, CT: AVI Publishing Company.

Bernfield P (1951). Enzymes of starch degradation and synthesis. Advances in Enzymology. 12: 379-481.

Deak T, Beuchat R (1987). Identification of food borne yeasts. J. Food Protect 50: 243-264.

Du Preez JC, Bosch MN, Prior BA (1987). Temperature profiles of growth and ethanol tolerance of the xylose fermenting yeasts *Candida shematae* and *Pichia stipitis*. Appl. Microbiol. Biotechnol. 25: 521-525.

Ekunsanmi TJ, Odunfa SA (1990). Ethanol tolerance, sugar tolerance and invertase activities of some yeasts strains isolated from steep water of fermenting cassava tubers. J. Appl. Bact. 69: 672-675.

Gray WD (1945). The sugar tolerance of four strains of distillers' yeast. J. Bacteriol. 49: 445-452.

Harrison JS, Graham JCJ (1970). Yeasts in distillery practice. In: The Yeasts Vol. 3 ed. Rose AH, Harrison JS. pp. 283-332. London Academic Press.

Jimenez J, Benitez T (1986). Characterization of wine yeasts for ethanol production. Appl. Microbiol. Biotechnol. 25: 150-154.

Kreger-van Rij NJW (Ed.) (1984). The Yeasts - A taxonomic study, 3rd edn. Amsterdam, Elsevier Science.

Layokun SK (1984). Use of the palm wine cultures for ethanol production from black strap molasses with particular reference to conditions in the tropics. Proc. Biochem. 19: 180-182.

Novak M, Stretiajano P, Moreno M, Goma G (1981). Alcoholic fermentation: Inhibitory effect of ethanol. Biotechnol. Bioeng. 23: 201-211.

Okafor N (1977). Microorganism associated with cassava fermentation for gari production. J. Appl. Bacteriol. 42: 279-284.

Okagbue RN (1988). A note on the leavening activity of yeasts isolated from Nigerian palm wine. J. Appl. Bacteriol. 64: 235-240.

Osho A, Odunfa SA (1999). Fermentation of cashew juice using the wine yeast strain NCYC 125 and three other isolated yeast strains. Adv. Food Sci. 21(½): 22-27.