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Ethanol production from native yeast isolated from local cultivars of *Musa sp*.

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Abstract

Ripe banana fruit is used to isolate and identify native yeasts from the different cultivars of *Musa sp.* such as Champa kola, Malbhog/Sapri kola, Saiel kola, Geera kola and Jahaji kola which can be utilized for the preparation of beverages yeast. Highest mean counts of yeast were 56.3 ×10^3 CFU/mL in the Champa kola of *Musa sp*. The total 44 yeast strains were identified, comprising ascomycetes and basidiomycetous with the most frequent being *Candida sp., Meyerozyma quilliermondii, Pichia quillerimondii* and *Saccharomyces cerevisiae* whereas *Cryptococcus sp*. was the most frequent yeast of basidiomycetous to be associated with the ripen banana pulp. The isolates were found potential to produce alcohol. Of the 28 isolates were fermented for 4 days in YEPD broth containing 400 g/L sucrose and 0.67 g/L yeast nutrient solutions. The alcohol content was measured in percent of the total volume. 12 yeast strains showed ethanol production ability. Two yeast strains *Phichia sp* and *Saccharomyces cerevisae,* was found to produce the most alcohol during the 3 day period (3.76% *v*/*v*,) whereas *Saccharomyces cerevisae* produced the least alcohol (1.31% *v*/*v*).

Key Words: Ethanol, Fermentation, *Musa* cultivars, Yeast strains

INTRODUCTION

Bananas (*Musa sp*) are major starch staple crops in the developing world. It is consumed as an energy yielding food and as a dessert. Bananas are consumed in ripe stage when it is yellow. The ripe fruits are processed into fermented or unfermented drinks and unripe ones are consumed as vegetable. Banana fruit is a rich source of nutrients and active compounds such as vitamin A, vitamin C, vitamin E, β-carotene, Phosphorus, Potassium, Calcium, Magnesium, Iron and Manganese. Fruits contain various antioxidants compounds such as gallocatechin (Someya *et al.*, 2002) and dopamine (Kanazawa and Sakakibara, 2000) which elevate blood pressure and stimulate the smooth muscle of the intestine.

From several centuries, yeast has been used in the production of food and alcoholic beverages, and today these organisms were used in a number of different processes in the industries. Fermentation is carried out in presence of various types of yeast. The yeast strains play a vital role in production of ethanol. Yeast including *S. cerevisiae* is a very attractive organism to work with since it is nonpathogenic, and due to its long history of application in the production of consumable products such as ethanol and baker's yeast, it has been classified as a GRAS organism (generally regarded as safe). Species not belonging to the *Saccharomyces* genus (non-*Saccharomyces* yeasts) introduce a certain dimension of ecological and biochemical diversity into winemaking presumably, this diversity is transferred to the organoleptic quality of wines (Fleet., 1993; Egli *et al*., 1998; Ferna´ndez *et al*., 1999). Deactivated yeast, usually *Saccharomyces cerevisiae* are rich source of protein and vitamins, especially the B-complex vitamins, minerals and cofactors required for growth (Centina-Sauri and Sierra Basto, 1994).

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). Many species of yeast are associated with fermentation of carbohydrates, flavour and quality by the production and excretion of metabolites during their growth and autolysis (Fleet, 1993, 2001, 2003; Lambrechts and Pretorius, 2000; Swiegers and Pretorius, 2005; Swiegers *et al*., 2005). These microorganism, are isolated from many fruits and other products for their desirable characteristics product with pleasant flavours. Thus the objective of this work was to isolate, yeast from different cultivars of *Musa sp*. and characterized in terms of its biochemical properties in order to obtain more information about the indigenous flora, and to select yeasts for ethanol production which is a prerequisite for biotechnological applications.

MATERIALS AND METHODS

Over ripe fruits of Champa kola (CK), Malbhog/Sapri kola (MK), Saiel kola (SK), Geera kola (GK) and Jahaji kola (JK) with large black spots of banana were collected from cultivated farm market of Silchar of Cachar district of South Assam. Bananas were collected aseptically in sterile polythene bags kept in a carton box, and transported to the Microbiology Laboratory of Assam University Silchar for further analyses.

Isolation of yeast isolates

10 g of fruit pulp of banana was mashed with 90 ml sterile distilled water. The puree was mixed thoroughly on a rotary shaker for 30 min and was shaken gently before use. This puree was

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serially diluted upto 10^{-5} times. Serial dilutions of the puree were made using one mililiter of the juice. Aliquots one millilitre of the dilution were plated on the yeast extract-malt extract agar (HiMedia M424,India) media, 0.5 g/L of streptomycin sulphate powder was incorporated as antibiotics to prevent bacterial growth. Control plating was carried out using sterile peptone water. The petriplates were incubated at 28 ± 2°C in the incubator for 72hrs (Ameh *et al.,* 1989). The colonies growing on these plates were counted to estimate the yeast population (CFU/mL). Individual colonies were picked up and transfer on fresh agar plates and pure cultures were transferred to YPD slants for 1 week and then recultured into fresh medium.

Identification and characterization of yeast isolates

The yeast isolates were identified using cultural and morphological characters. Identification procedures were done according to standard methods described in Barnett *et al.*, (1990). The physiological and biochemical test like assimilation of some nitrogen compound, cycloheximide resistance, Urea hydrolysis, nitrate utilization, sugar utilizing test, were carried, vegetative structures were observed using single stain or counter staining (Gilman, 1971). Species names followed by the suffix -like indicate that the organism is similar to this species but is sufficiently different to be considered a probable new species

Screening of yeast for alcohol producers

The strains were examined for their tolerance to ethanol by culturing them on YPD agar plates containing 10% alcohol. Those strains that could grow on the alcohol plates were screened further by culturing them in YPD broth containing 10% ethanol and potassium meta-bisulphate (0.2%). Samples were withdrawn after 24, 48 and 72 hr and the growth data was analysed by spectrophotometer at 660 nm.

Estimation of alcohol

A method based on that Caputi *et al*., (1968), was performed to estimate the alcohol percentage. Sample (2 mL) was taken in 50mL distilled water in a distillation flask and was distilled at 65°C. 15 mL of the distillate was collected in a conical flask and chromic acid (25 mL) was added. Volume made up to 50 mL with distilled water. The conical flasks were incubated at 60°C on a water bath for 30 minutes. The solutions were brought to room temperature and the optical density was read at 600 nm spectrophotometrically. The alcohol percentages of the samples were calculated using the standard graph produced using absolute alcohol in the range of 0-12% and subjected to the same conditions of distillation and estimation.

RESULTS AND DISCUSSION

Isolation, Identification and characterization of yeast isolates

The pH of the banana samples was found to be around 4.5-5.0 which are slightly acidic in nature due to high organic acid content (Bharti Vyas *et al*., 2007) as shown in Fig. 1. It was observed that in summer season, the highest pH was recorded in JK cultivar and lowest in MK. In Monsoon season, the highest pH was recorded in JK cultivar and lowest in MK whereas in winter season, the highest pH was recorded in JK cultivar and lowest in CK during whole year.

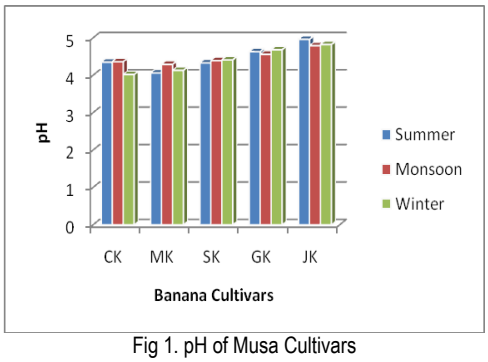


Fig 1. pH of Musa Cultivars

The moisture content of a fresh fruit is related to its dry matter content and therefore the loss of moisture in April was 78% in SK was much lesser compared to others cultivars. In Monsoon, highest moisture loss occurred in MK (Fig. 2).

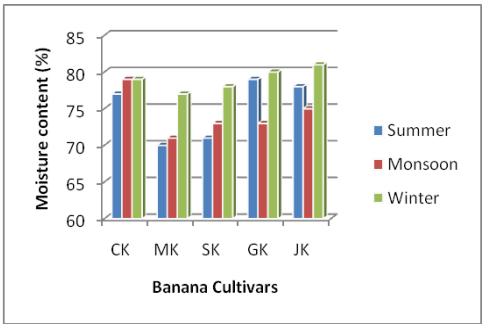


Fig 2. Moisture loss in pulp during drying

At the end of drying period 1-3 g dry material was obtained. This shows that among these cultivars of banana JK and GK has highest moisture content followed by CK, SK and MK in winter season.

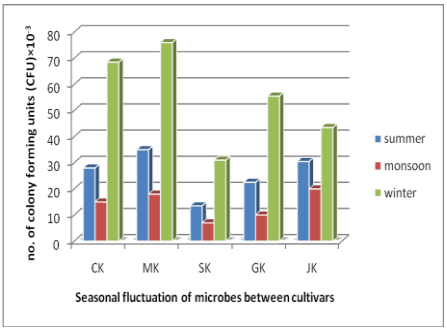


Fig.3. Seasonal effect on CFUof *Musa* cultivars

Figure 3 shows that the total yeast counts were in the range of 7 ×10^3 to 68 ×10^3. Highest yeast count on YPD agar plates was observed in CK 56.3 ×10^3 CFU/mL in winter season whereas the lowest yeast count was in observed in monsoon in SK cultivars 7×10^3 CFU/mL. The isolations made in winter season 0.56% (CK), 8.36% (MK), 2.11% (SK), 3.58% (GK) and 3.74% (JK) in rainy season 0.95% (CK), 7.13% (MK), 5.08% (SK), 5.71% (GK) and 3.21% (JK) yeast fungi where as in summer season, 0.93% (CK), 2.81% (MK), 1.83% (SK), 2.22% (GK) and 2.62% (JK) including

Table 1. Biochemical properties of yeast strains showing highest ethanol yield

No		Characteristics		
1		Cell morphology	Ellipsoidal	Oval
2		Cell size	l=4.8; b=2.1	l=14.3: b=4.8
3		Mycelium	True, pseudo	Pseudo
4		Ascospore	Globose	Hat shaped
5		Growth at 37°C	+	+
6		Nitrate reduction	-	-
7	Sugar ferment	Glucose	+	+
8		Galactose	+	+
9		Lactose	-	-
10		Maltose	+	+
11		Raffinose	+	+
12		Sucrose	+	+
13		Starch	+	-
14		Trehalose	-	-
15	Sugar Assimilated	Arabinose	-	+
16		Celliobiose	-	+
17		Galactose	+	+
18		Glycerol	+	+
19		Inositol	-	-
20		Lactose	-	+
21		Maltose	+	+
22		Mellibiose	+	-
23		Mannitol	-	+
24		Raffinose	+	+
25		Rhamnose	-	-
26		Sucrose	+	+
27		Starch	+	+
28		Trehalose	+	+
29		Xylose	-	-
		Identify	YC-5 (*S.cerevisiae*)	YC-9 (*Pichia sp.*)

C.glabrata, Debaryomyces hensenii, and *C.latennulate* which was not detected in rainy and winter season in another cultivars (Fig. 4) . A similar dominance was reported by Davenport (1976) and Blake man (1985) in apple, grapes and on the phylloplane of broad beans.

Of 97 yeast isolates 58 were identified into 10 genera and 44 were identified into 38 species with *Pichia*, *Candida sp* and *Saccharomycopsis* being the most frequently encountered genera. The yeast species identified were *Candida ciferrii* (2), *C. glabrata* (3), *C.catenulata* (1), *C. lunata* (1), *Saccharomyces cerevisae* (8), *Torulopsis sp* (1), *Candida parapsilosis* (8), *C. fukuyamaensis* (1), *C. albicans* (3), *Cryptococcus sp* (1), *Debaryomyces hensenii* (2,) *Pichia quilliermondii* (6),*Rodoturula rubra* (1).

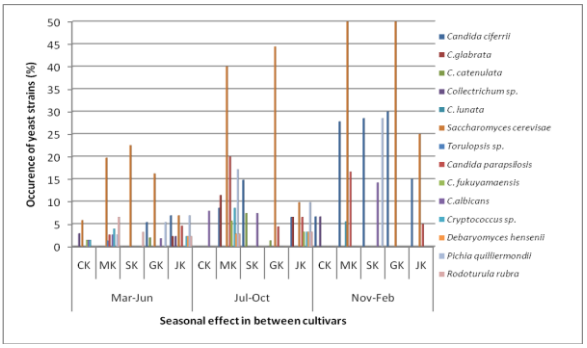
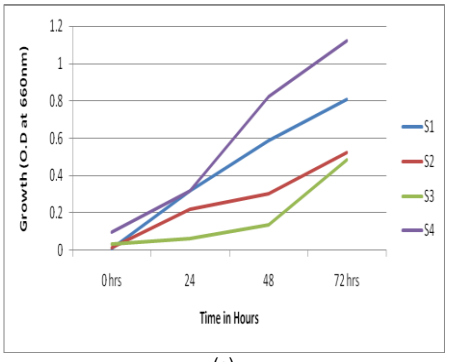


Fig.4 Occurrence percentage of yeasts species of *Musa* cultivars.

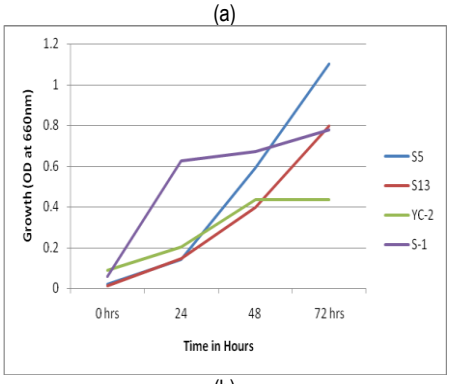
Winter season supported the greatest diversity 61.88% and the most frequent yeast included *Saccharomyces cerevisae* and *Debaryomyces hensenii*, the least number of yeast supporeted in summer season including *C.lunata, Collectrichum sp* and *Torulopsis sp*. The yeast fungi from the cultivars more than 10% occurence and above were *S.cerevisae,* and *Rodoturula rubra while* the yeast component with less than 10% occurence was *Candida catenulate, Candida ciferrii, Candida parapsilosis, C. fukuyamaeensis, C. albicans, and Pichia quilliermondii.* The yeast fungi were isolated from the pulp of bananas shows a close observation with the mycoflora isolated from black plum (Okigbo, 2001). It was shown that the frequency of occurrence of *Saccharomyces cerevisae* and *Pichia quilliermondii* were found to be higher as compare to *C. catenulate, C. glabrata, Debaryomyces hensenii*, and *Torulopsis sp.* The others diversity is decreased due to *Saccharomyces sp* attain high occurrence (frquency), this high numbers signifies dominance of a single species. This could be attributed to enzymatic breakdown of starch to sugars by the fungi (Ogundero, 1978). Species of yeast (*Saccharomyces cerevisae* and *Candida sp*.) were found in our study matched those of Ethiraj and Suresh (1988) on mangoes.

Yeast for ethanol tolerance

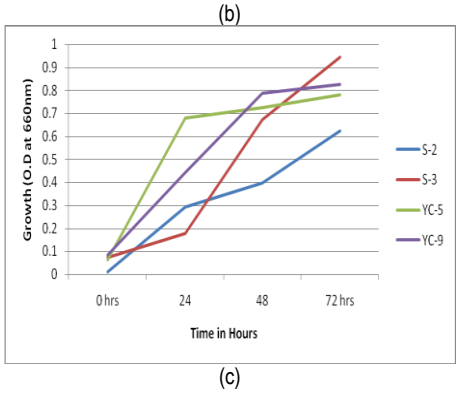
A total of 12 yeast strains were selected based on their ethanol tolerance, estimating their growth in presence of exogenously added ethanol (10% *v/v*) to YEPD media (Fig. 5 - a;b;c).



(a)



(b)



(c)

Fig 5. (a), (b), (c) Growth patterns of selected yeasts in presence of ethanol

Two of the twelve isolates were able to grow in 10% (*v*/*v*) ethanol concentration and above. In Fig 6. S5 showed less tolerance to ethanol than the other species of the *S. cerevisiae.* All the two strains namely YC-5 (NCBI Accession no: JN093146) and YC-9, of the *Pichia sp*. showing highest alcohol percentage. Yeast species that produce low concentration of ethyl alcohol might have another role in contribution of desirable flavor of the products.

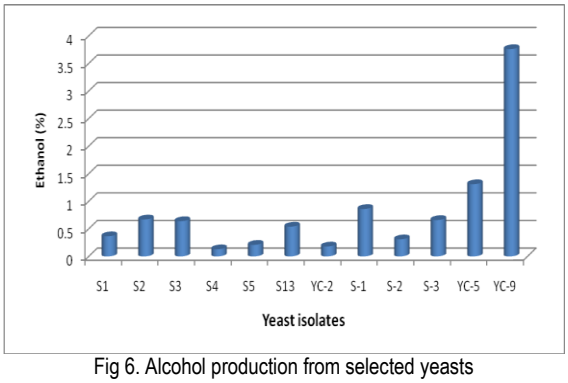


Fig 6. Alcohol production from selected yeasts

Mazimum alcohol producing strain were biochemically charecterized which shown in Table 1. Strain isolated from Malbhog/Sapri kola it was revealed that their is variation in alcohol percentage was 3.76 and 1.31 respectively. The highest growth rate was shown by the strain S-3. The least alcohol percentage was found in S4.

CONCLUSION

The results in this study suggest that the yeasts are associated with the ripened banana pulp, almost all have been isolated previously from soil, on fruits and trees (Kurtzman and Fell, 1998) and all are with good fermentation attributes and producing alcohol. In Assam banana are available in abundance as a raw material for isolation of ethanol tolerant yeast. Two yeast strains namely YC-5 and YC-9 were isolated from Sapri/Malbhog kola (*Musa sp*.). Although *S. cerevisiae* and *Pichia* ethanol yield is low, but the strains could be genetically manipulated for higher ethanol yield.

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