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Microbial Enumeration and Analysis of Antioxidant Activity of Starter Cultures Used for Rice Beer Preparation Unique to Some Ethnic Communities of Assam, India

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ABSTRACT

Consumption and use of traditional rice beers in socio-cultural and religious activities amongst different ethnic communities of Assam and other states of north-east India are known for generations. Apart from socio-cultural and religious relevance, traditional rice beers are believed to have significant nutritive and health benefits. The rice beers of *Deori*, *Mising* and *Ahom* communities are known as *Sujen*, *Apong* and *Xaj* respectively while the local names of the starter cultures are called *Sujen fero*, *Apop* and *Xaj pitha* respectively. The microflora present in dried starter tablets converts starchy materials to fermentable sugars and subsequently to alcohol and organic acids. The antioxidant activity of rice beers may be derived from the different plant ingredients used for preparation of starter cultures. Analysis of microbial population and antioxidant activity of starter cultures are essential as quality of rice beer is dependent largely on the raw materials used. A comparative study on the microbial population and antioxidant activity of the starter cultures used for rice beer preparation of three ethnic communities of Assam, viz. *Deori*, *Mising* and *Ahom* communities was done. In YEPDA medium, yeast counts (log CFU g^{-1}) for starter culture samples (in 10^{-3} dilution) are found to be in the range of 6.5-6.6. Radical Scavenging Effects of *Sujen fero* and *Xaj pitha* are high,i.e., 80.63% and 87.69% as compared to *Apop*(55.47%). The total phenolic content of *Apop* was found to be 31.94 and that of *Sujen fero* and *Xaj pitha* are 17.96 and17.80 respectively. The starter culture samples displayed positive antioxidant activity making it healthy for consumption.

Keywords: Rice beers, Starter culture, Deori, Mising, Ahom, Antioxidant activity, Radical scavenging effect

INTRODUCTION

In the North-Eastern region of India, rice beer consumption and its preparation is a popular practice among the ethnic communities. [1,2] It is an integral component in the socio-cultural life of the tribal people. The rice beers are prepared by fermentation of rice grains with natural yeast in the form of starter cakes. The use of rice wine starters is believed to have originated in China and gradually it spread to other countries of Asia. Starter cultures are known by different local names such as *banh men* in Vietnam, *chu* in Chinese, *koji* in Japanese, *nuruk* in Korean, *murcha* in Indian, *ragi* in Indonesia, *ragi tapai* in Malaysia and *bubod* in Philippines. [3] The starter cakes used in the North-Eastern region of India consist of a consortium of different groups of microflora such as moulds, yeast and lactic acid bacteria. [4-7] The use of these kinds of mixed cultures for fermentation contributes to the formation of various esters and alcohols. [8] The choice of starter cakes influences the yield and quality of the rice beer. The microflora present in dried starter tablets convert starchy materials to fermentable sugars and subsequently to alcohol and organic acids. [9-12] Rice beers of North-East India including Assam exhibit antioxidant activities as reported by Bhuyan *et al.* [13] The antioxidant activity may be derived from the different plant ingredients used for preparation of starter cultures. Analysis of

microbial population and antioxidant activity of starter cultures are essential as quality of rice beer is dependent largely on the raw materials used. [14]

We are reporting herein a comparative study on the microbial population and antioxidant activity of the starter cultures used for rice beer preparation of three ethnic communities of Assam, viz. *Deori*, *Mising* and *Ahom* communities. The rice beers of *Deori*, *Mising* and *Ahom* communities are known as *Sujen, Apong* and *Xaj* respectively while the local names of the starter cultures are called *Sujen fero, Apop* and *Xaj pitha* respectively.

MATERIALS AND METHODS

Sample collection

Starter culture samples of each community were collected from three different villages of Sivasagar district of Assam, India. They were stored in an air-tight container until use.

Microbial enumeration of starter cultures of rice beers

The starter cultures (SC) prepared by three communities, viz. starter cultures by *Deoris* (SCS), by *Misings* (SCA) and by *Ahoms* (SCX) residing in Sivasagar district of Assam, India were analyzed for the presence of yeasts and bacteria.

One gram of finely pounded SC of each community was subjected to serial dilution technique and spread plate method for the detection of microbial strains and calculation of total colony formed. [15] Media used were yeast extract peptone dextrose agar (YEPDA) for yeasts and nutrient agar (NA) for bacteria. Inoculation was done using 10 µL of each of 3 selected dilutions, in duplicate and spread with sterile L-spreader. The plates were then incubated at 30 ºC for 24 h. After 24 h, microbial colony forming units (CFU) were counted. CFU per gram of the sample can be calculated as follows:

$$\frac{CFU}{g} = \frac{colonies\ on\ plate \times dilution\ factor}{inoculum\ volume}$$

The results are expressed in terms of log of colony forming units per gram of sample. Biochemical tests were routinely done following the initial morphological identification of the cultures on agar media and microscopy.

Evaluation of Antioxidant activities of starter cultures using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

Preparation of starter culture (SC) extracts

The ethanol extracts of the starter cultures (SC) were prepared according to Soares *et al.* with some modification. [16,17] Prior to extraction, the dry starter cakes of each variety were powdered to fine forms using mortar and pestle. 4 g of the dry powder was weighed accurately and each sample was mixed with 40 mL of ethanol in a round bottomed flask (which was wrapped with aluminium foil to prevent exposure to light). The mixture was magnetically stirred for 24 h at room temperature. Thereafter the SC extract was filtered through a Buchner funnel, and the filtrate was evaporated to dryness in a rotary vacuum evaporator (Buchi rotavapour, R 200) at 40 ºC. Solid extracts were refrigerated and protected from light (by covering with an aluminium foil) until analyzed.

DPPH *Assay*

The solid SC extract was first diluted with ethanol to obtain 10 mg in 1 mL solution. The DPPH Assay was done to measure the antioxidant activity. The DPPH solution (100 µM) was prepared fresh in ethanol. DPPH ethanolic solution was mixed with diluted SC extract in 1:1 ratio. The mixture was incubated in dark for 15 min at room temperature. Subsequently the absorbance of the mixture was measured at 517 nm on a UV spectrophotometer. The antioxidant activities are expressed in terms of radical scavenging effects of DPPH and also in terms of Equivalent Vitamin C Antioxidant Activity (EVCAA).

Determination of total phenolic content (TPC) of starter culture (SC) extracts

Folin-Ciocalteu method with slight modification was applied for determining

the TPC of SC extracts. The solid SC extract was first diluted with ethanol to obtain 10 mg in 1 mL solution. An aliquot of 0.5 mL of diluted SC extract was mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent. The mixture was shaken and allowed to react for 4 min. Then 2 mL of aqueous Na_2CO_3 solution (7.5% w/v) was added, and the final volume was made up to 10 mL with distilled water. After 2 h of incubation in dark at room temperature, the absorbance was measured at 765 nm against the blank reagent. The measurements were calibrated with a standard curve of gallic acid (GA) solution. The TPC of the starter cultures are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (dw) of SC extract (mg of GAE/g of dw).

RESULTS AND DISCUSSION

Microbial counts of starter cultures

The starter cultures (SC) of the three rice beers, namely *Sujen*, *Apong* and *Xaj* labeled as SCS, SCA and SCX respectively, were investigated for the presence of yeasts and bacteria. These starter cakes, being rich sources of various micro-organisms, can prove to be useful in food industries. The approximate number of yeasts and bacteria present per gram of SC sample are presented in Table 1 and Table 2. The count of yeasts and bacteria observed in the SC samples are given in Table 3. Isolated colonies of yeast and bacteria are shown in Fig. 2 and Fig. 1.

Table 1: Total colony count of yeasts in different starter cultures

Sample code	Dilution factor	CFU	Inoculum Vol.	Approx. No. of yeasts present per gram
SCS	10^{-3}	32	10 µL	32×10^5
	10^{-4}	15		15×10^6
	10^{-5}	9		9×10^7
SCA	10^{-3}	35		35×10^5
	10^{-4}	20		20×10^6
	10^{-5}	12		12×10^7
SCX	10^{-3}	38		38×10^5
	10^{-4}	16		16×10^6
	10^{-5}	11		11×10^7

SCS=Starter culture for *Sujen*, SCA= Starter culture for *Apong*,
SCX= Starter culture for *Xaj*
(Values are the means, n=2).

Table 2: Total colony count of bacteria in different starter cultures

Sample code	Dilution factor	CFU	Inoculum Vol.	Approx. No. of bacteria present per gram
SCS	10^{-4}	59	10 µL	59×10^6
	10^{-5}	40		40×10^7
	10^{-6}	13		13×10^8
SCA	10^{-4}	50		50×10^6
	10^{-5}	35		35×10^7
	10^{-6}	20		20×10^8
SCX	10^{-4}	48		48×10^6
	10^{-5}	31		31×10^7
	10^{-6}	15		15×10^8

SCS=Starter culture for *Sujen*, SCA= Starter culture for *Apong*,
SCX= Starter culture for *Xaj*
(Values are the means, n=2).

Table 3: Microbial counts (log CFU g^{-1}) of the SC samples

Sample code	Yeast count (log CFU g^{-1})	LAB count (log CFU g^{-1})[a]
SCS	6.5	7.8
SCA	6.5	7.7
SCX	6.6	7.7

SCS=Starter culture for *Sujen*
SCA= Starter culture for *Apong*
SCX= Starter culture for *Xaj*
[a]LAB refers to Lactic Acid Bacteria

In YEPDA medium, yeast counts (log CFU g^{-1}) for SC samples (in 10^{-3} dilution) are found to be in the range of 6.5-6.6. The strains were identified based on cultural, morphological and biochemical characteristics (carbohydrate assimilation and fermentation test, utilization of ethanol as carbon source). Biochemical tests were routinely done following the initial

morphological identification of the cultures on agar media and microscopy. A total of 20 yeasts and 35 lactic acid bacteria (LAB) were isolated from the three samples. From 10 colonies studied, 6 colonies showed all features of *S. cerevisiae* species. When tested about the fermentation ability, 7 strains showed fermentation of most of the tested carbohydrates. Table 4 shows the result of fermentation test. D-glucose, sucrose, maltose and D-galactose were fermented but lactose was not. Ethanol utilization was tested and the result obtained followed the pattern of *Saccharomyces cerevisiae*. The dominant strain in all the samples is identified as belonging to the genus Saccharomyces and closely resembles *Saccharomyces cerevisiae*. Other strains identified are Candida *sp.* in SCX and Rhodotorula *sp.* in SCS. Reported literatures reveal the presence of strains like *Saccharomyces cerevisiae,* strains of *Candida* species like *C. krusei, C. pelliculosa, C. utilis, C. sphaerica, C.magnolia* and *Rhodotorula glutinis*. [18]

Lactic acid bacteria (LAB) are a group of fermentative bacteria and are abundant in environments rich in nutrients where carbohydrates and proteins are usually present. They have remarkable selective advantages in diverse ecological niches due to the efficient use of nutrients and the production of lactic acid during growth. [19] The LAB are found in good numbers in all of the SC samples. Lactobacillus *sp.* is found to be the dominant species. Presumptive LAB was further selected based on the morphology, Gram reaction and catalase test. Strains of Enterococcus *sp.,* Bacillus *sp.* and Staphylococcus *sp.* are also observed. The presence of *Lactobacillus plantarum* and *L. brevis* are reported earlier as the major lactic acid bacteria in starter cultures. [20] All the three SC samples have similar number of LAB count (log CFU g^{-1}) of 7.8 for SCS and 7.7 for both SCA and SCX. Tamang *et al.* [11,21] have reported the average population of LAB (log CFU g^{-1}) in *hamei* (starter cake used in Manipur, India) to be 6.9 and in *marcha* (starter cake used in Sikkim, India) to be 7.1. Seemingly better population of LAB in the three samples, namely SCS, SCA and SCX, is indicative of their potential as sources of probiotic organisms.

TABLE 4: Carbohydrate fermentation test

Carbon Sources	Positive control	STRAIN									
		01	02	03	04	05	06	07	08	09	10
Glucose	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	–	+	+	+	+	+
Maltose	+	+	–	+	–	–	+	+	+	+	+
Galactose	+	+	+	+	+	–	+	+	+	+	+
Lactose	–	–	–	–	–	–	–	–	–	–	–

Assimilate carbon source (+); do not assimilate carbon source (–);
positive control (*Saccharomyces cerevisiae*).

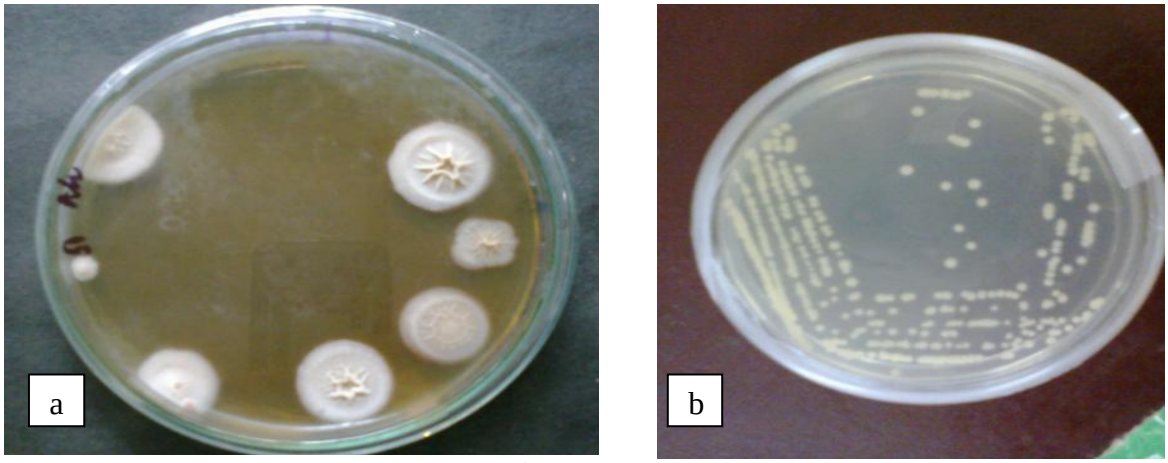

Fig 1: Isolated colonies of a) Yeast and b) Bacteria on agar plates.

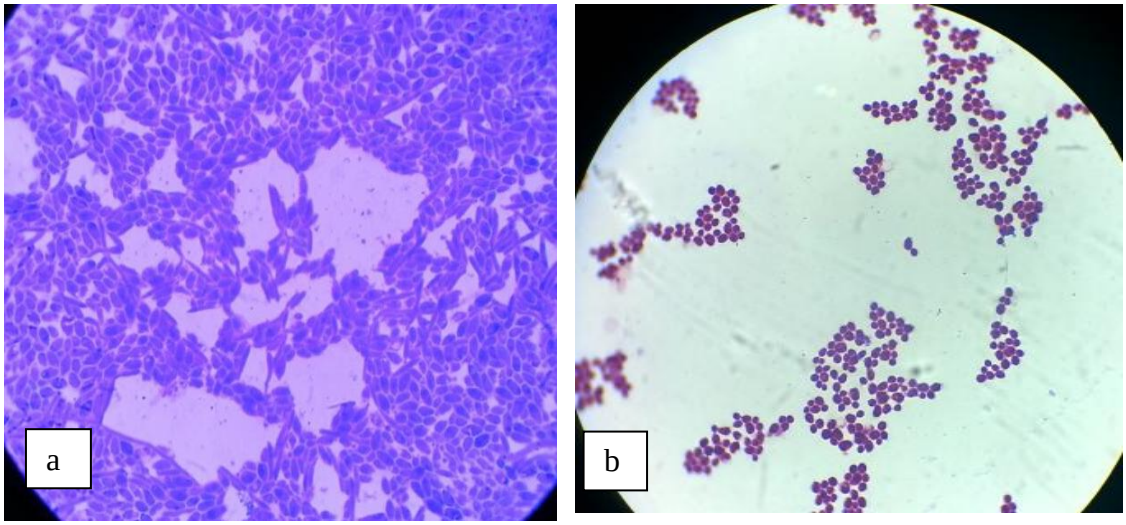

Fig 2: Yeast strain under microscope (100x) a) Sachharomyces *sp*, b) Candida *sp*.

Radical scavenging effect (RSE) of the starter cultures (SC)

Radical scavenging effects (RSE) of the starter culture (SC) extracts are shown in Table 5. RSEs of samples SCS and SCX are high as compared to SCA.

Table 5: Antioxidant activity of SC samples measured by DPPH assay

Sl. No.	SC type	Wt. of one SC (g)	Amount of dry wt. (mg) extracted from 4 g SC	RSE%	EVCAA (mmol/L)
1	SCS	15	198.2	80.63	82.85
2	SCA	11	149	55.47	55.35
3	SCX	36	179.5	87.69	90.56

Values are means of three replicate measurements

The total phenolic content (TPC) of the starter cultures (SC) are shown in Table 6.

Table 6: Total phenolic content (TPC) of the starter cultures (SC)

Sl. No.	SC type	TPC (mg GA/g dw SC)
1	SCS	17.96
2	SCA	31.94
3	SCX	17.80

Values are means of three replicate measurements

CONCLUSION

While it is generally accepted that the choice of the starter culture strongly influences the yield and quality of the beer, there is not much knowledge of the relationship between the microbial composition of starters and their performance. The limited knowledge about traditional starter cultures poses an obstacle to industrial development and, thus, these starters have attracted the attention of researchers in food microbiology and technology, and in studies concerning the selection of safe and storable superior starters for small-scale fermentation processes. The starter culture samples displayed positive antioxidant activity making it healthy for consumption. So these can be utilized as a functional food due to its high bioactive potentials.

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