



ISOLATION OF YEAST AND MOLD FROM BODO AND KARBI RICE BEVERAGES

Microbiology

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ABSTRACT

India is the second largest country in population and there are numerous numbers of tribes and tradition among it. They all have different habits. Northeast is among the main area of tradition. There are various people of different cast lived in Northeast region. But the livelihood, culture, language religion of each tribe is found different from each other. Similarly Bodo and Karbi of Assam are two tribes whose socio-cultural practices are different. Both the Bodo and Karbi tribes prepare their traditional fermented alcoholic beverage for this respective social and religious functions and festivals. This beverage is a fermented product made of rice and rice starter cake. The rice starter cakes contain various microorganisms which help in fermented rice beverage production. The present study was undertaken for isolation of yeast and mold from two starter cakes, rice beverage and rice. The aim of the present work was to isolate yeast and mold and study the different isolates on the basis of their morphological and biochemical characteristics. Sensory evaluation of beverage and estimate the alcohol content of beverage was also done on this study. The isolate strain was identified as *Saccharomyces* on the basis of sugar fermentation and *Rhizopus* on the basis of microscopic observation.

KEYWORDS

Starter cake, Rice beverage, Carbohydrate fermentation

INTRODUCTION :-

India is an agricultural country. It produces large numbers of fruits and vegetables. From cereals, various types of beverages are produced countrywide. In Assam also, beverages are produced from rice from ancient times. There are many traditional ways to make these beverages (Basumatary *et al.* 2014). In various traditions, peoples produces different beverages (like Jou, Apong, Laopani, Hor alank) from rice (Pegu *et al.*, 2013). In these beverages various yeast and moulds are present which helps to ferment the beverages.

Fermentation is a viable technique in the development of new products with modified physicochemical and sensory qualities especially flavour and nutritional components. Alcohol, acetic and lactic acid fermentations are important for quality in production. Out of these, alcoholic fermentation is widely employed for the preparation of beverages in which alcohol is major constituent. Microorganisms used for fermentation transform raw minerals into biochemically and organoleptically useful products with the harmful products like phytales, tannins and polyphenols being destroyed or detoxified. Every single fermented food is synergy with distinctive group of micro flora which increases the level of proteins, vitamins, essential amino acids, fatty acids and other nutritional constituents (Narzary *et al.*, 2016).

Rice wine from glutinous rice is a popular traditional alcoholic beverage in North-Eastern states of India. It is manufactured under non-sterile conditions at home scale using traditional solid rice flat cakes as starter. The principle of rice wine manufacturing consist of the saccharification of steamed rice starch by fungal enzymes under aerobic solid state fermentation and the moulded mass is mixed with water and is allowed to undergo submerged alcoholic fermentation by yeasts using traditional starter flat cakes (Jeyram *et al.*, 2008). Rice wine is produced by amylolytic process, which is the conversion of starch into sugars by the action of acids or enzymes like amylase (Subhsree, 2010).

The starter cake is the indispensable part of rice wine preparation. The people of all communities use the starter cake for preparation of their traditional beverage. But, it is seen that method of preparation of said starter cakes are different according to respective tribes. They contain yeasts, lactic acid bacteria, amylolytic microorganisms and starch degrading molds. The microbial content and rice variety which is used also contribute to the different taste and flavour of these beverages. The quality of the starter cakes depend upon the variety of plant parts used, rice variety used and maintenance of hygienic and sanitary conditions. (Senapati and Gurmeyum., 2016). In Starter Cakes preparation various medicinal plants used such as *Polygonum glabrum*, *Ananas comosus*, *Artocarpus heterophyllus*, *Piper nigrum*,

Clerodendrum viscosum, *Adhatoda vasica*, *Accacia pennata*, *Asparagus racemosus*, *Centella asiatica*, *Cinnamomum tamala*, *Gomphostemma parviflora*, *Costus speciosus*, *Vitex negundo*, *Leucas plukenetii* etc.

An alcoholic beverage is a drink that contains ethanol. These are divided into three general classes for taxation and regulation of production namely beers, wines, and spirits distilled beverages such as whisky, rum, gin, vodka etc. Beer is made by fermentation of starch combining yeast and malted cereal starch, especially barley corn, rye, wheat or blend of several grains and usually flavoured with hops. It contains 4 to 8 per cent alcohol and its energy value ranges between 28 and 73 kcal per 100 mL. Distilled alcoholic beverages are produced by distilling ethanol by fermentation of grains, fruits or vegetables. They are made from sugarcane juice, molasses, fermented mash of cereals and potatoes and fermented malt of barley and rye. The alcohol content in distilled alcoholic beverage ranges between 40 and 60 per cent (Swami *et al.*, 2014)

In north east India various types of beverages are prepared by the tribal people such as Poro, Horo, Judima and Jumai by Misshing, Karbi, Dimasa and Bodo people respectively. The local tribal wine show more protein, carbohydrate than foreign one (Arjun, 2015).. It also plays an important role in the socio-cultural life of the tribal people as it is found to be associated with many occasions like merry making, ritual ceremonies, festivals, marriages and even death ceremonies. The preparation and consumption of this type of liquor emerged mainly due to the climatic conditions and discovering the use of surrounding natural resources. There are also reports of rice beer being used as a drug. It works effective against insomnia, headache, body ache and inflammation of body parts, diarrhoea and urinary problems, expelling worms and as a treatment of cholera (Das *et al.*, 2012).

There are many advantages, uses of beverages of Assam. Therefore, there is the interest in the study of microorganisms present in fermented rice beverages of Assam. Investigation of the microbes will help to standardised production parameters and lead to production of consistent quality end products. Thus study has been designed with the objectives of study some selected isolates of yeast and mold from Bodo and Karbi rice beverages.

Thus keeping in view the importance of yeast and mold, the present study has been designed with the following objectives.

1. Isolation of different strain of yeast and mold.
2. Biochemical characterization, microscopic and macroscopic studies

METHODOLOGY :

The research work entitled "Isolation of Yeast and Mold from Bodo

and Karbi rice beverage" was carried out in the Microbiology Laboratory of department of Microbiology, College of Allied Health Science, Assam downtown University, Guwahati.

1.1 SAMPLE COLLECTION

Samples (starter cake) were collected from two tribes (Bodo and Karbi) of Guwahati region mainly Beltola and Hengrabari.



Fig : Starter cakes

1.2 ISOLATION OF YEAST AND MOLD WITH STREAK PLATE METHOD

Isolation was done by using streak plate method on Sabouraud Dextrose Agar media (SDA). The medium was sterilized by autoclaving at 121 °C for 15 mins. The sterilized agar medium was cooled and added 2ml antibiotic (Amoxicilline) solution and after that poured into sterile petridishes and allowed to solidify. One loopful of the sample was withdrawn and the inoculum was placed on the agar surface and streaking was done from side to side in parallel lines across the surface of area. The plates were inoculated in inverted position at 28°C for 24 hours.

1.3 MACROSCOPIC STUDY

Morphology of yeasts and molds and their appearance on SDA media was examined based on their cultural characteristics (colony form, elevation, margin, appearance, pigmentation, optical property and texture).

1.4 MICROSCOPIC STUDY OF YEAST BY WET MOUNT TEST

A clean slide was taken. Put a drop of water is placed in the middle of the slide. Cell was picked up from the plate with a sterilized inoculating needle and mixed properly with water. A clean cover slip is placed carefully over the slide by avoiding the formation of bubbles. The preparation was observed under microscope at 40x magnification.

1.5 MICROSCOPIC STUDY OF MOLD BY LACTOPHENOL COTTON BLUE STAINING

The lactophenol cotton blue (LPCB) is also a wet mount test, widely used for the staining and observation of fungi. It is a semi-permanent microscopic preparation technique of fungi. The LPCB stain contains three components. They are phenol (kill any live organism), lactic acid (preserves fungal structures), cotton blue (stains the chitin and cellulose of the fungal cell wall intensely blue).

A clean glass slide is taken. After that put a drop of LPCB is placed in the middle of the slide. Sample was picked up from the plate with a sterilized inoculating needle and mixed properly with LPCB. A clean cover slip placed carefully over the slide by avoiding the formation of bubbles. The preparation was examined under the microscope first under 10x followed by 40x magnification.

1.6 BIOCHEMICAL CHARACTERISTIC

Various biochemical tests such as carbohydrate fermentation, ethanol tolerance, estimation of alcohol are performed for the identification of various isolates.

1.6.1 CARBOHYDRATE FERMENTATION

A metabolic process performed by almost all type of organism is known as fermentation. These will result in the formation of ATP. In positive test, production of acid and gas, the broth turned in to yellow colour. In negative test, no colour change in the test tube.

Fermentation broth was prepared and taken in test tubes. Insert inverted Durham tubes into all tubes, the Durham tube should be fully filled with broth. Broth taken into fermentation tubes was autoclaved at 15 lb pressure for 15 minutes. The tubes were then inoculated with the isolates and incubated for 24-48 hours. The tubes were observed for acid and gas formation.

1.6.2 ETHANOL TOLERANCE TEST

Ethanol tolerance test was performed by preparing glucose (2%) broth

containing YEP (1%) and phenol red. Broth taken is fermentation tube was autoclaved at 12lb pressure. Ethanol solution of three different concentrations (12%,14%,16%,18%) was prepared and added to the sterilized tubes (4 concentrations for each strain). The tubes were then incubated with the yeast strain and incubated for 24-48 hours. The tubes were observed for acid and gas formation.

1.7 PROCEDURE OF RICE BEVERAGE PRODUCTION

We take 250 gm of bora rice and aijung rice and boiled it separately. After boiled, separate the water from rice and allowed it to dry. Now two ankur powdered mixed with rice and put inside the plastic bag and kept for one night. Next day mixture was transferred into 4 different earthen pots and added some water and left covered with banana leaves for 3-4 days. After 3-4 days, rice beer is prepared.

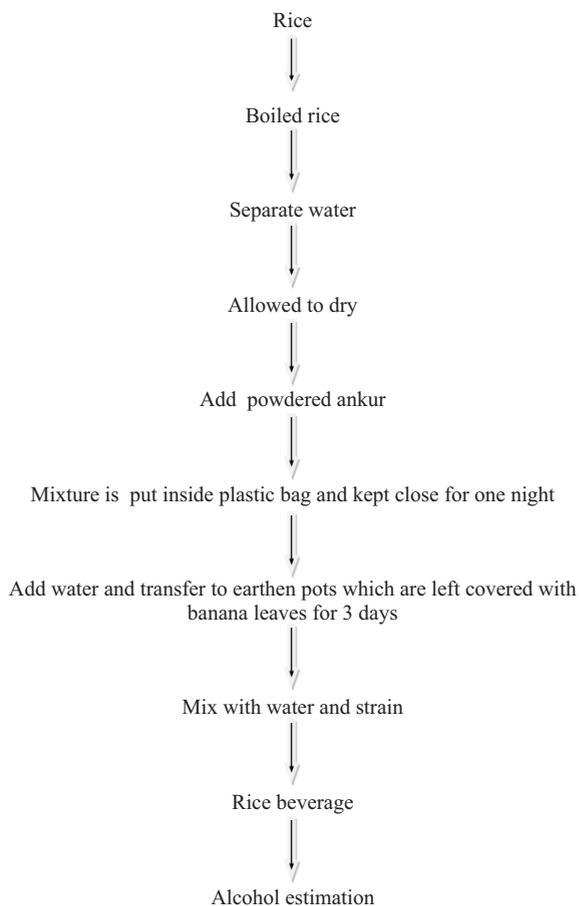


Fig : (A) boiled aijung rice (B) boiled bora rice



Fig : (C) Prepared Bodo and Karbi rice beverage

1.8 ESTIMATION OF ALCOHOL

The method of estimating ethanol in alcohol consists of colour reaction of ethanol with sodium dichromate. The colorimetric quantification is based on the formation of green coloured chromate ions resulting from

treatment of ethanol and sodium dichromate as limiting reactant in presence of sulphuric acid and acetate buffer pH 4.3. The absorbance maxima for the ethanol were found to be 578 nm. The influence of acetate buffer pH reaction time, and Beer's law on colour development and sensitivity were investigated and optimal assay conditions established.

Concentration of ethanol was calculated by using following formula (Sandip et.al, 2016),

$$\text{Percentage of ethanol in sample (\%)} = (C_s / C_u) (A_u / A_s) \times 100$$

where C_s = Concentration of standard, C_u = Concentration of sample as per Labelled Claim, A_u = Absorbance of standard, A_s = Absorbance of sample,

1.9 SENSORY EVALUATION

Sensory evaluation of rice beverage (taste, smell, texture, appearance) was done by panel of 5 judges.

RESULTS AND DISCUSSION :-

In this study, samples were collected and from these samples yeast and molds are isolated. Yeast and mold are dominating microorganisms which are mainly present in fermented beverage. Various microorganisms are present in fermented food and beverages. Several genera with hundred species of yeast have been isolated from fermented foods and alcoholic beverages, which mostly include *Candida*, *Debaryomyces*, *Geotrichum*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Saccharomycopsis*, *Schizosaccharomyces*, *Torulopsis*, *Wickerhamomyces* and *Zygosaccharomyces* (Tamang et.al, 2013). Species of *Actinomucor*, *Amylomyces*, *Aspergillus*, *Monascus*, *Mucor*, *Neurospora*, *Pencilium*, *Rhizopus* and *Ustilago* are reported for many fermented foods, and alcoholic beverages (Chen et.al 2014).

In Bodo and Karbi rice beverage, dominant flora in yeast and mold. 14 yeast isolates and 5 mold were obtained from sample. The yeast isolates were characterized on the basis of microscopic observation, carbohydrate fermentation and ethanol tolerance test.

Ethanol tolerance test and carbohydrate fermentation test were used on the different isolates in order to have a better understanding of their behaviour. The isolated yeast were tested to ferment glucose, sucrose, lactose, maltose and mannitol and to produce carbon dioxide. Result showed that all isolates were able to ferment only glucose and sucrose and some can ferment maltose also and they releasing carbon dioxide gas as observed in tube. The isolate does not ferment lactose and mannitol. This is an important strain for strain used in alcohol production.

One of the importance characteristic of microorganism which are utilized for the industrial production of alcoholic beverage is that these microorganism should have high ethanol capacity, i.e, it can survive and produce ethanol even in presence of ethanol (Shah, 2012). In this study, the isolates were checked for their tolerance capacity and we found that some of these isolates can tolerate ethanol concentration at 12%, 14%, 16%. The isolates were inhibited by 18% ethanol. These results suggest that these isolates have good ethanol tolerance capacity and thus can be industrial importance.

Sensory evaluations of beverage are also done by panel of 5 judges. They give their opinion about taste, texture, appearance and smell.

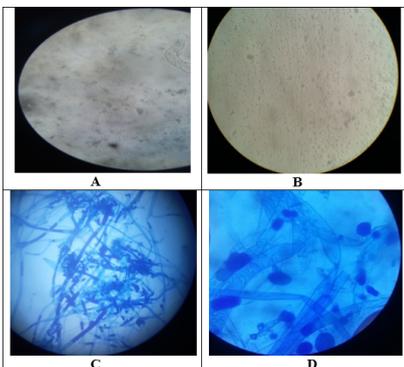


Fig 1 : (A) B(I) yeast isolate microscopy from starter cake ; (B) B(ii)

yeast isolate microscopy from starter cake ; (C) Mold (10X) microscopy from B starter cake ; (D) Mold (40X) microscopy from B starter cake.

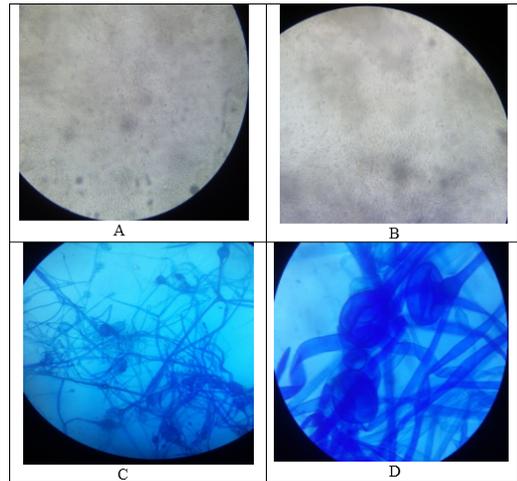


Fig 2: (A) Microscopy of K(I) yeast from starter cake ; (B) Microscopy of K(ii) yeast from starter cake ; (C) Microscopy of mold (10X) from starter cake ; (D) Microscopy of mold (40X) from starter cake

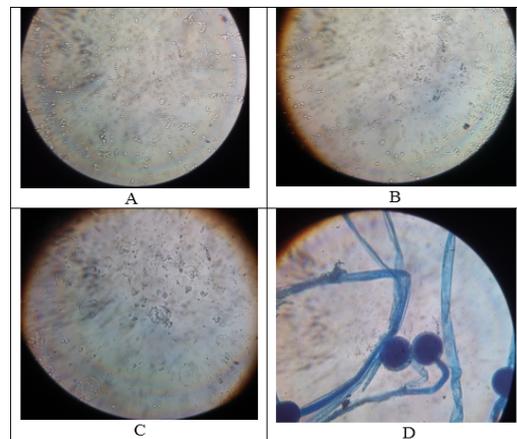


Fig 3 : (A) Microscopy of B(B) yeast 1 from rice beverage ; (B) Microscopy of B(B) yeast 2 from rice beverage ; (C) Microscopy of B(A) isolate from rice beverage ; (D) Microscopy of mold (40X) from B(A) rice beverage

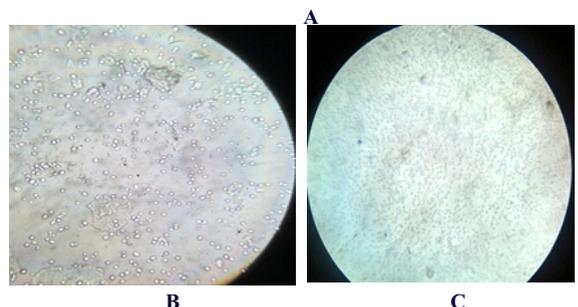


Fig 4: (A) Microscopy of yeast isolate from K(A) rice beverage ; (B) Microscopy of K(B) yeast 1 from rice beverage ; (C) Microscopy of K(B) yeast 2 from rice beverage

Table 1:- Carbohydrate fermentation test of yeast isolate from starter cakes

	Glucose	Sucrose	Lactose	Maltose Mal	Mannitol
B (i)	+	-	-	+	-
B(ii)	+	+	-	+	-
K(i)	+	+	-	+	-
K(ii)	+	+	-	+	-

Table 2:- Carbohydrate fermentation test of yeast isolate from rice beverage

	Glucose	Sucrose	Lactose	Maltose Mal	Mannitol
K (A)	+	+	-	+	-
B(A)	+	+	-	-	-
B(B)	+	+	-	-	-
K(B) 1	+	+	-	-	-
K(B)2	+	+	-	-	-

Table 3:- Carbohydrate fermentation test of yeast isolate from rice

	Glucose	Sucrose	Lactose	Maltose Mal	Mannitol
K (A) R	+	+	-	-	-
B(A) R	+	+	-	+	-

Table 4:- Ethanol tolerance test for yeast isolate from starter cake

	12%	14%	16%	18%
K(i)	Acid formation (high turbidity)	Acid formation (moderate turbidity)	Acid formation (low turbidity)	-
K(ii)	Acid and gas formation (high turbidity)	Acid formation (moderate turbidity)	Acid formation (low turbidity)	Low turbidity
B (i)	-(no turbidity)	-(no turbidity)	-(no turbidity)	-(no turbidity)
B (ii)	-(no turbidity)	-(no turbidity)	-(no turbidity)	-(no turbidity)

Table 4:- Alcohol content of rice beverage

Beverage	Alcohol content	pH
B(A)	6.36 %	5
B (B)	7.22%	5
K (A)	5.35%	5
K (B)	7.94%	5

CONCLUSION :-

In the North East region, among each tribe varieties are found in their ethnic fermented food products. Their fermented food microorganisms are also observed with its special diversities. Microbial diversity ranges from filamentous molds to enzyme producing to alcohol producing yeasts, and gram positive and few gram negative bacteria with biological function.

From the study entitle "Isolation of yeast and mold from Bodo and Karbi rice beverage" it could be concluded that 14 yeast and 5 molds are isolates from beverages and their starter cakes, which help in fermentation process.

From this study on Carbohydrate Fermentation, it is known that all the isolate can ferment glucose and sucrose and some of them can ferment maltose also.

From ethanol tolerance test, it is known that some of these isolates can tolerate ethanol concentration at 12%, 14%, 16%. And these isolates are inhibited by 18% ethanol. The beverage is analysed and pH is found around 5.

This study also confirmed that this two beverage may provide suitable environment and source of nutrient for the growth yeast with potential fermentation properties. This fermentation have low cost of production as it need less labour input and the raw material required for fermentation and product development are easily or locally available. The finding of the present investigation could be an important step towards the possibilities of using these strains in industrial purposes.

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