

Original Research Article

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Identification of Yeast Strain Isolated from Nduiyi of Dima Hasao District of Assam, India

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ABSTRACT

Keywords

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Nduiyi is a traditional amylytic starter used to produce sweet-sour alcoholic beverage, called Nduijao by Jeme Naga in Dima Hasao (formerly, North Cachar Hills) district of Assam. The aim of this study was to examine the Yeast flora present in Nduiyi collected from Dima Hasao district of Assam. Total 4 samples of Nduiyi was collected from Jeme Naga villages of the Hill district. Based on cell morphology and Phenotypic characterization isolates were identified as *Candida glabrata* and *Saccharomyces cerevisiae*. It was hypothesized that both the groups of Yeast have a primary role in alcohol fermentation.

Introduction

Nduiyi is a traditionally prepared mixed amylytic dough inocula used as a starter by the Jeme Naga tribe of Dima Hasao district of Assam for the production of indigenous alcoholic beverage the Nduijao. During the traditional method of preparation, bark of *Albizia myriophylla* Benth. (Family - Mimosaceae) or leaves of *Saccharum officinarum* L. (Family - Poaceae) is used. These are dry, flat- rounded and oval, creamy white to dusty white, solid starter ranging from 1.1 cm to 10.1 cm in diameter with the weight ranging from 15 g to 25 g. The method of preparation is as follows: Sticky rice is soaked for 9-10 h at room temperature and crushed with bark of *Albizia myriophylla* or

leaves of *Saccharum officinarum* and 2-3 % of previously prepared Nduiyi as mother culture. The mixture is then made into paste by adding water and kneaded into flat rounded and oval cakes of varying sizes and kept it for one to three days at 25-30° C and sun dried for two to three days. Finally, cakes are stored in a dry place for more than a year. Nduiyi is also similar to traditional mixed amylytic starters such as humao of Dima Hasao (Chakrabarty *et al.*, 2014), marcha of Sikkim (Tamang and Sarkar, 1996) &, ragi of Indonesia (Saono *et al.*, 1974).

The present study investigated the Yeast present in Nduiyi of Dima Hasao district and

their possible roles in production of popular indigenous alcoholic beverages of the Hill district.

Socio-economy

Nduiyi is produced at home and is sold in local periodical markets exclusively by the rural women. Cost of a single *Nduiyi* (size, 15-25 g) is around Rs.10-20/ depending on size.

Ethnic value

In any religious festivals, the ethnic people offer *Nduiyi*(*starter*) to each male and female in the name of family God and Goddesses.

Materials and Methods

Collection of Samples

A field survey was conducted in different Jeme Naga villages of Dima Hasao district. Different samples of *Nduiyi* were collected from different Jeme Naga villages as well as local market. All samples were collected aseptically in pre-sterile poly-bags transported to laboratory for analyses.

Isolation of yeasts

Ten g of sample were homogenized with 90 ml of 0.85 % (w/v) sterile physiological saline in a stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution (10^{-1} to 10^{-8}) in the same diluent was made.

Enumeration and isolations were targeted for Yeast. Yeasts were isolated on yeast-malt extract (YM) agar (M424, HiMedia), supplemented with 10 IU/ml benzylpenicillin and 12 µg/ml streptomycin sulphate, respectively; and plates were incubated aerobically at 28° C for 72 h (Tsuyoshi *et al.*, 2005).

Morphological characterisation

Morphological studies of yeast isolates were performing using yeast –malt extract (YM) agar and YM broth (Difco Laboratories), incubated at 25 ° C for 72 h. To induce the sporulations of yeasts, acetate agar, corn meal agar, Gorodokowa agar, 5% malt agar, potato –dextrose agar, vegetable juice agar, and YM agar were used according to Yarrow (1998).

Physiological and biochemical characterization

Physiological and biochemical characteristics of the yeasts isolates were determined according to the API test (API 20C AIX; BioMerieux S.A., Marcy- 1'Etoile, France) and the method described by Yarrow (1998).

Ethanol production

The ethanol yield of yeast isolates was determined after growth at 25° C for 4 d in YM broth containing 10% glucose. Ethanol concentration in the culture supernatant was determined by the enzyme assay (F-Kit Ethanol; Roche Diagnostics K.K., Tokyo, Japan).

Results and Discussion

Nduiyi is used as a starter for the preparation of *Nduijao* (ethnic fermented beverages of Dima Hasao used by Jeme Naga. It has been observed that Yeast present in the starter were high in numbers. It is exclusively prepared by the rural women belonging to the Lodi, Asalu, Nutan Gunjung, Boro haflong, Lisong basti of Dima Hasao.

Based on morphological and physiological characterization the yeast strains were identified Different samples of *Nduiyi* (mixed amylolytic starter) of Dima Hasao district of

Assam were analysed for Yeast (Table 1) population. The average yeast population in Nduiyi were found at the level of 10^8 Log cfu/g in all the samples, lactic acid bacteria was comparatively less ($\sim 10^5$ cfu/g), *Bacilli* were at the level of 10^6 cfu/g, and mould at the level of 10^7 cfu/g. Filamentous moulds were not recovered in any finish product of Nduijao indicating that moulds have roles only in the initial phase of fermentation mostly in saccharification of the substrates. The P^H of Nduijao (alcoholic beverage) was 4.3 ± 0.1 , titratable acidity was 0.44 ± 0.01 and alcohol percentage was 4.0-6.0. Mineral (copper, chromium, manganese, iron, zinc, magnesium, potassium, sodium, lead, nickel and selenium) present in uncooked rice and in fermented beverage *i.e.* Nduijao was estimated. The result showed higher content of minerals in fermented beverage than that of raw substrate.

The morphological and physiological characteristics identified the isolates as *Saccharomyces cerevisiae* & *Candida glabrata*. Amylolytic and alcohol-producing yeast isolated from *humao* was *Saccharomyces cerevisiae* (Chakrabarty *et al.*, 2014).

In this study, phenotypic studies of the Yeasts isolated from Nduiyi demonstrated that the amylolytic starters constituted more diverse microflora than previously recognised. The yeast species identified were *S. cerevisiae* and *C. glabrata*. The identified yeast species, *S. bayanus* and *C. glabrata* were also encountered in and isolated from several other Asian amylolytic starters (Deak, 1991; Hadisepoetro *et al.*, 1979; Hesseltine *et al.*, 1988; Hesseltine and Kurtzman, 1990).

Table.1 Microbial populations of Nduiyi (mixed amylolytic starter) and Nduijao, fermented alcoholic beverage of Dima Hasao district of Assam

Product	Collection	LAB Log cfu/g	Bacilli Log cfu/g	Yeast Log cfu/g	Mould Log cfu/g
Nduiyi	Haflong bazaar (n = 2)	5.9 ± 0.06	6.8 ± 0.0	8.2 ± 0.06	7.2 ± 0.0
	Lodi basti (n = 2)	5.8 ± 0.0	6.8 ± 0.06	8.3 ± 0.0	7.3 ± 0.06
Nduijao	Haflong bazar (n = 2)	5.2 ± 0.06	6.8 ± 0.06	7.5 ± 0.0	NIL
	Laisong basti (n = 2)	5.2 ± 0.06	6.8 ± 0.06	7.4 ± 0.06	NIL

n = number of samples. Data represents the means (\pm SD) of samples.

Table.2 Identification of yeasts isolated from Nduiyi

Isolate code	Cell size (µm)	Ascospore	Growth at 37°C	Sugars Fermented								Sugars Assimilated											Identity					
				Glucose	Galactose	Lactose	Maltose	Raffinose	Sucrose	Starch	Trehalose	Arabinose	Cellobiose	Galactose	Glycerol	Inositol	Lactose	Maltose	Melibiose	Mannitol	Raffinose	Rhamnose		Sucrose	Glucose	Trehalose	Xylose	
DHN:Y1	l= 4.5 (3.2 - 5.4) b=2.8 (1.6 - 4.7)	Globose	+	+	+	-	+	+	+	+	-	-	-	+	+	-	w	+	+	-	+	-	+	w	+	-	<i>Saccharomyces cerevisiae</i>	
DHN:Y2	l= 4.7 (3.2 - 5.6) b= 2.4 (1.6 - 4.8)	Globose	+	+	+	-	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	-	+	+	+	+	-	<i>Saccharomyces cerevisiae</i>
DHN: Y3	l = 3.5 (1.6 -4.0) b=2.8 (1.5 - 3.2)	Globose	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	-	-	-	+	w	-	<i>Candida glabrata</i>	
DHN:Y4	l=3.7 (1.5-4.2) b=2.5(1.2-3.3)	Globose	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	w	-	-	-	-	+	+	-	<i>Candida glabrata</i>	

All yeast isolates were oval to ellipsoidal in shape, produced pseudo-mycelium l, length; b, breadth; w, weak, +, positive, -, negative. DHN stands for Dima Hasao Nduiyi, Y for Yeast isolate.



(a) J. Chakrabarty taking a interview with a Naga woman in a Naga village of Haflong town (Dima Hasao)



(b) Naga woman selling Nduijao in Haflong bazaar, Haflong (Dima Hasao)

Fig. 2 Naga woman selling Nduijao in Haflong bazaar, Haflong (Dima Hasao)



Fig. 3 Starter ready for sale.



Saccharomyces have a primary role in alcohol fermentation. *C. glabrata*, non-*Saccharomyces* yeast is a moderate alcohol producer, which has also been recovered from Nduijao, indicating that it is involved in alcohol production. May be non-*Saccharomyces* yeast might contribute to flavour or aroma formation in the alcohol beverage (Rojas *et al.*, 2001) (Table 2). This starter making technology reflect the traditional method of subculturing desirable inocula from previous batch to new culture using rice as base substrates. Nduijao the popular drink is believed to be good tonic for ailing persons and post-natal women also. Because of high calorie, they consume the popular drink Nduijao to regain the strength.

Minerals content between fermented alcoholic beverage and cooked rice were also checked. Higher minerals content in Nduijao indicate contribution of mineral intake in the daily diet of the local people of the hill district. Yeasts and molds play a major role in the fermentation of foods during which there is bio-enrichment leading to the production of proteins, vitamins, minerals, aroma, alcohols, acids, esters and also improvements in digestibility, preservation, and organoleptic properties. May be, because of the presence of yeasts and Lactic Acid Bacteria (LAB) present in the Fermented foods and alcoholic beverages, the Ethnic foods are safe to eat.

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