



RESEARCH ARTICLE

Bacterial population from plant-based traditionally processed foods of Tripura, India

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Abstract

This present study was aimed at processing four ethnic vegetable foods in laboratory condition and enumeration of bacterial population dynamics in terms of CFU/g counts along with characterization of bacterial isolates. CFU/g was enumerated daywise from these foods. The isolated bacteria were characterized biochemically and with nucleotide analysis. The study includes two fermented foods namely *melye amiley* and *midukeye*, *muya rukjak* as boiled bamboo shoot and one sun dried vegetable, *mulai kwrn*. The selected food items generally take one to three days of fermentation and processing prior to consumption. The study of the bacterial population during the tenure of fermentation revealed that in *melye amiley* and *midukeye* were maximum (7.45 and 8.77 log CFU/g, respectively) in the second day of fermentation and then declined (6.58 and 7.81 log CFU/g, respectively) in the third day. The six isolates belonging to two genera of bacteria were identified from four ethnic foods. Out of the six bacterial isolates of ethnic foods, two from each *midukeye* and *mulai kwrn* whereas one each from *melye amiley* and *muya rukjak* were isolated. This study highlights information regarding bacterial loads associated with the food items.

Keywords

bacterial characterization; log CFU; fermented food; traditional knowledge

Introduction

Traditional foods are prepared by indigenous people and modes of preparations of these are unique for each of the indigenous communities that passed down from generation by generation. Traditional foods include fermented food and these food products are preferred over conventional food items due to their unique taste, palatability and delicacy. Fermentation involves the production of a food item with the help of microorganisms and the indigenous people have been using these microbes naively for various purposes (1). Fermented edible products are prepared from raw or cooked materials of animal and plant origin by using micro-organisms either spontaneously or by adding mixture or by pure culture (2, 3). These cost-effective traditional fermented foods are produced by the involvement of microflora and bio-chemical reactions or the enzymatic actions (4-6).

The ethnics women's wisdom and indigenous skill extent from cultivation to harvesting of agricultural products, fermentation to culinary skills and its production to its marketing for local economy subsistence was greatly appreciable and a matter of praise (7). The women of these regions have played an indispensable role as they have the knowledge of fermenting the seasonally available food items and conservation of these food items for future use and passed down the valuable information from one generation to another (8). Locally available materials like milk, vegetable, bamboo,

soybean, meat, fish and cereal are commonly fermented by the ethnic people for using during unfavourable conditions (9, 10).

Tripura, a state of the northeastern region of India covers 10,491 sq. km area. A total of 19 different ethnic groups inhabits the states, and most of them are Kokborok speaking people. The indigenous people of this state are very much fond of eating and preparing ethnic dishes. They are actively involved in processing of food as these are an integral part of their custom.

Many fermented foods were reported from Northeast India (10). The traditional food habits amongst the ethnic population of Tripura are very simple but differ from tribe to tribe (11). Uchoi et al. (11) also reported certain traditional foods of Tripura. Despite numerous literatures available from the northeastern states of India, the elaborate works related to this field is still lacking from Tripura. However, the knowledge on microbial flora inhabiting these foods of Tripura is absent. Therefore, paucity of such kind of study promoted us to process some traditional foods in laboratory condition and to enumerate the microbial population, in addition we have characterize selected bacterial isolates associated with these food items.

Materials and Methods

Preparation of traditional processed foods under laboratory condition

The field surveys were conducted in different villages and markets of Tripura during the year 2016 (March-September) for gathering information related to the traditional foods used by the ethnic communities. The location of the villages and markets along with the plant parts used as raw materials are depicted in Table 1. A total of six markets and seven villages were considering for this present study. The survey was conducted among the three different ethnic communities viz., Debbarma, Chakma and Jamatia. The information gathered on indigenous food items preparation processes from the forest dwellers and villagers were noted down and further verified from interviews with the elderly persons and women who are actively involved in the preparation of food items. The number of informants varies from 30-45 depending on their presence during the survey. The information regarding the preparation of traditional food was employed for processing in the Laboratory of the parent Institute. The temperature and relative humidity recorded during the study were $32\pm 2^{\circ}\text{C}$ and $78\pm 4\%$ during the processing of these traditional foods.

The raw materials used for the preparation of the foods were procured from the local village markets and transported to the laboratory after zipping them in the marked sterile polythene bags for further analysis and preparation.

The processing in the laboratory condition was done following the information described by the ethnic people. The respective ethnic communities that consumed these food items are presented in Table 1.

Enumeration of the bacterial population

For the enumeration of bacterial population nutrient agar (NA) media was used. After inoculation of 1 g of raw and processed food in the NA media, the Petriplates were incubated for 24-48 hrs at 37°C followed by the enumeration of bacterial populations after serial dilution (up to 10^6). The bacterial population was recorded as CFU/g of samples and then converted to logarithmic scales as log CFU/g. For the bacterial population dynamics study, the enumeration was conducted after one day of fermentation subsequently for 3 days in *melye amiley* and *midukeye*. In *muya rukjak*, a population study was done for one day after boiling as this food item can be consumed on the next day of preparation. The enumeration of bacteria from *mulai kwrn* was done during the storage at room temperature after drying for one day which was considered as processed sun-dried food.

Characterization of selected bacterial isolates

The dominant six bacterial isolates from four ethnic foods were selected based on their colony morphology. The maximum colonies having the same morphological characteristics and appearance were considered as the prevalent one and considered for biochemical and molecular characterization after isolation from the study of the population. A total of nineteen biochemical tests were performed for each of the six bacterial isolates to characterize them biochemically. All the biochemical tests were performed for the bacterial isolates (12).

Molecular characterization of selected bacterial isolates

The genomic DNA was extracted using Gene Elute Genomic DNA isolation kit (Sigma) as per the instructions of the manufacturer and served as a template for PCR. Each reaction solution contained approximately 2.5 mM MgCl_2 , 10 ng of DNA, 2 pmol of each forward and reverse primers, 1x PCR buffer (Bangalore Genei, Bangalore, India); 200 μM each dCTP, dGTP, dATP and dTTP and 1 U of Taq DNA polymerase (Bangalore Genei, Bangalore, India) in a final volume of 20 μl . To amplify 16S rRNA gene, 27F and 1492R primers were used as reported earlier (13). Polymerase Chain Reaction was performed using Eppendorf Gradient Mastercycler system with a cycle of 94°C for 3 min; 32 cycles of 94°C for 45 sec, 51°C for 1 min and 72°C for 1.30 min and a final extension at 72°C for 10 mins, and the reaction mixture was kept at 4°C . PCR products were sequenced using ABI PRISM 3100 Genetic Analyzer. The sequence was submitted to NCBI and accession numbers were obtained.

Table 1. Plants and their parts used for the preparation of fermented food items from Tripura, Northeast India

Food items	Plants used	Family	Place of collection	Ethnic group	Parts used
<i>Melye amiley</i>	<i>Melocanna baccifera</i> (Roxb.) Kurz	Poaceae	Machmara	Chakma	Shoot
<i>Midukeye</i>	<i>Melocanna baccifera</i> (Roxb.) Kurz	Poaceae	Machmara	Chakma	Shoot
<i>Mulai kwrn</i>	<i>Raphanus raphanistrum</i> L.	Brassicaceae	Dainmara para	Debbarma	Tap root
<i>Muya rukjak</i>	<i>Bambusa balcooa</i> Roxb.	Poaceae	Bagma	Jamatia	Shoot

Results

Mode of processing of foods

Melye amiley

The tender bamboo shoots were washed in running tap water followed by washing with distilled water. The tender bamboo shoots were cut into approximately 0.5 cm in thickness and allowed to dip in water (Fig. 1a) in a local made earthen pot or any other utensils having a tight lid. In this manner, it was kept for two to three days and has a sour taste. Then it was ready to cook.

Midukeye

Tender bamboo shoots were first cleaned and washed several times with running tap water and cut transversely (approximately 0.5 cm in thickness) and wrapped tightly with the banana (*Musa* sp.) leaves (Fig. 1b). This preparation was

kept in moist places for 2-3 days. After 2-3 days this food product is cooked for consumption.

Mulai kwran

Fresh (radish) was collected from local markets and washed thoroughly to remove the adhering soil particles and organic debris followed by cutting transversely at approximately 0.5 cm in thickness. Then the slices of radishes are sun-dried for one day (Fig. 1c). The dried samples were now ready to store for future use. The sun-dried samples were stored in local made earthen pot or glass jars.

Muya rukjak

The tender bamboo shoots were cleaned, washed and cut transversely into small pieces (approximately 0.5 cm in thickness). These small slices of bamboo shoots were boiled in water for approximately 1 hour (Fig. 1d). The duration of the boiling period depends on the species of bamboo shoots used



Fig. 1. Ethnic foods of Tripura: (a) Bamboo shoots immersed in water for preparation of *melye amiley*. (b) Bamboo shoots in banana leaf for fermentation of *midukeye*. (c) Tap root of radish after sundried ready for storage of the product, *mulai kwran*. (d) Bamboo shoots after boiling for preparation of *muya rukjak*.

for preparation. These boiled bamboo slices could be eaten after processing.

The fermented food products are consumed by the ethnic people of Tripura in their daily recipe as they believe that these food items are highly nutritious although the modes of preparations are different. These traditional foods are prepared from different phytoresources by adopting the traditional knowledge which reaffirmed with the earlier findings (15). The types of plant products used in ethnic food productions depend on the availability of the desired plants (16). The ethnic peoples of this particular region used different vegetables and bamboo shoots during the preparation of these food products which corroborates with the previous findings (10, 17). The preparation method of *Miya mikhri* of Dimasa tribes of Assam (10, 18) is similar to *midukeye* of Chakma community of Tripura.

Naturally fermented foods and beverages include both functional and non-functional microorganisms (19, 20). Functional microorganisms are capable of renovating the chemical constituents of raw materials during food fermentation resulting in the enhancement of bio-availability of nutrients, the sensory quality of the food, bio-preservative effects and improvement of food safety, degradation of toxic components and anti-nutritive factors, production of antioxidant and antimicrobial compounds, stimulation of the probiotic functions, and fortifying with some health-promoting bioactive compounds (7, 21-23). To date people have been following the traditional methods for fermented food preparations and the food products often contain mixed microbial populations because of lack of sterility and the use of natural fermentation (24).

Bacterial population dynamics during fermentation

The daywise population growth during the tenure of the fermentation process was evaluated and it was evident that in *melye amiley* and *midukeye* the bacterial population were maximum (7.45 and 8.77 log CFU/g, respectively) during the second day of fermentation and then declined in the third day (6.58 and 7.81 log CFU/g) (Table 2). The bacterial population was lower in raw form of *melye amiley* and *midukeye* (5.82 and 5.88 log CFU/g, respectively) as compared to fermented ones. However, in raw form of *mulai kwan* and *muya rukjak* harbours a higher population (7.60 and 6.82 log CFU/g, respectively) compared to the processed product.

The higher population in *mulai kwan* in raw form could be attributed to the fact that the population was assessed before sun-dried condition and subsequently decreased after sun drying. The higher bacterial population in raw form was observed in *muya rukjak* may be since the population was assessed before boiling of the shoots and after

boiling the population was found to be less.

The bacterial population associated with these ethnic food products is in agreement with the earlier finding (26). In this present study, the daywise bacterial population growth was enumerated till the foods were ready for consumption. In the fermented food items such as *melye amiley* and *midukeye*, the bacterial populations were maximum when inoculated during the second day of fermentation and declined on the third day. The possible cause for such a reduction in the bacterial population growth may be due to the emergence of biochemicals from the food items.

Identification and characterization of selected bacterial isolates

The biochemical characteristics of bacterial isolates are presented in Table 3. A total of 19 tests were performed for six bacterial isolates.

The six isolates identified to be from two genera (Table 4). Both genus were characterized by employing biochemical and molecular methods. AML01, MUL01, MUL02, BAM01 and BAM02 were identified as *Staphylococcus sciuri* and MRJ01 to be *Serratia marcescens* based on 16S rRNA analysis. The number of bases in the sequence of the bacterial strains AML01, MUL01, MUL02, BAM01, BAM02 and MRJ01 were 1002, 960, 960, 1093, 981 and 987, respectively. The Genbank accession and their isolation source are presented in Table 4.

All the foods were mainly vegetables, ethnic foods products which have been inhabited by the same bacteria. The reason may be due to laboratory conditions which have limited various bacterial species to inhabit and be involved in the process of preparation. *Staphylococcus sciuri* has been reported from various fermented foods (26-27) which agrees with this study. *Serratia* sp. was also reported from food items (27). The bacteria recovered from these food items needs to be tested for the fermentative ability along with sensory quality of the foods.

Conclusion

This study is an effort to process traditional food items along with the population count and characterization of the bacterial isolates from four ethnic foods. The bacterial population changed from raw to processed foods and differs during processing. The study was conducted from processed foods done in laboratory condition, however, the isolation and characterization from the foods directly from the local houses may harbour different bacterial flora. Further studies on the nutritive and medicinal properties of these processed

Table 2. Bacterial population (log CFU/g of samples) from raw and during fermentation, and processing and bacteria isolated (laboratory collection number) under laboratory condition

Food items	Microbial population (log CFU/g)				Bacterial isolates
	Raw	Fermented and processed			
		1 st day	2 nd day	3 rd day	
<i>Melye amiley</i>	5.82	6.56	7.45	6.58	AML01
<i>Midukeye</i>	5.88	7.82	8.77	7.81	BAM01, BAM02
<i>Mulai kwan</i>	7.60	6.80	6.69	6.70	MUL01, MUL02
<i>Muya rukjak</i>	6.82	5.00	-	-	MRJ 01

Table 3. Biochemical characterization of selected bacterial isolates isolated from fermented and processed food items

Tests	Bacterial isolates					
	AML01	MUL01	MUL02	BAM01	BAM02	MRJ01
Gram staining	+	+	+	+	+	-
Acid fast staining	-	-	-	-	-	-
Negative stain	+	+	+	+	+	+
Capsule stain	+	+	+	+	+	+
Spore stain	+	+	+	+	+	+
Methyl Red test	-	-	-	-	-	+
Voges-Proskauer test	-	-	-	-	-	-
Nitrate reduction test	+	+	+	+	+	-
Starch hydrolysis	+	+	+	+	+	+
Lipid hydrolysis	-	-	-	-	-	-
Casein hydrolysis	-	-	-	-	-	+
Indole test	-	-	-	-	-	-
Citrate	-	-	-	-	-	-
Urease	-	-	-	-	-	-
Catalase	+++	+++	+++	+++	+++	++
Oxidase	+	+	+	+	+	+
Phenyl alanine	-	-	-	-	-	-
Gelatin	+	+	+	+	+	-
Hydrogen sulfide production	-	-	-	-	-	-

Table 4. GenBank accession and source of bacteria isolated from traditionally processed foods of Tripura

Bacteria	GenBank accession	Isolation source	Laboratory collection number
<i>Staphylococcus sciuri</i>	MN630850	Fermented bamboo shoot	BAM01
	MN630852	Processed radish	MUL01
	MN630855	Fermented bamboo shoot	AML01
	MN630854	Processed radish	MUL02
	MN630849	Fermented bamboo shoot	BAM02
<i>Serratia marcescens</i>	MN630851	Boiled bamboo shoot	MRJ01

food products may aid crucial information to use these products widely not only pertaining to Tripura but to other places.

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Authors' contributions

The present study was designed by PD. The samples were collected by BD. BD, ARD and KC performed experiments and handled the research data. Data analysis was conducted by BD, ARD, KC and PD. PD, KC and AKS drafted the manuscript. Finally, all the authors approved and submitted the manuscript.

Compliance with ethical standards

Conflict of interest: No conflict of interest was declared by the authors.

Ethical issues: None

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