Antimicrobial, antioxidant and probiotics characterization of dominant bacterial isolates from traditional fermented fish of Manipur, North-East India

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Abstract *Utonga-kupsu*, *Hentak* and *Ngari* are traditional fermented fish products produced by the Manipuri people living in the North-Eastern part of India. The present study was designed with the aim to isolate, identify and characterize the microorganisms present in these fermented foods. Bacterial pure cultures were isolated using serially diluted samples and were further identified by conventional biochemical tests and Sanger sequencing of 16s rRNA gene. Results show that the number of bacterial count in Nutrient agar and Starch casein agar was 14–20 and 10–16 CFU/g, respectively. A total of 46 morphologically different bacterial strains were identified and assigned under the phylum Firmicutes. Identified bacterial strains belonged to the genus *Bacillus* and *Staphylococcus* and majority of the isolates were *Bacillus subtilis* and *Staphylococcus nepalensis*. Bacterial isolate HNS60 isolated from *Hentak* and identified as *Bacillus subtilis* was shown to possess high antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Most of the identified bacteria were shown to possess DPPH radical scavenging as well as phosphatase, amylase, protease and cellulose activities. The isolate HNS60 contain high antimicrobial, enzymatic and probiotics activity which might responsible for the possible health benefits of the fermented foods. *Utonga-kupsu*, *Hentak* and *Ngari* thus can

be further exploited as a potential source of probiotics and natural antioxidants.

Keywords Fermented fish · 16s rRNA · Antimicrobial · Enzymatic activities · Probiotics

Introduction

Fermentation, an economical and oldest method, is used for food processing and preservation (Tamang et al. 2012, 2016). Traditionally fermented foods have been consumed widely since it contains several beneficial activities. It is often used for probiotic purposes by introducing beneficial bacteria into the digestive system and helps in balancing of bacteria of the digestive system; for better absorption of the nutrients in the foods by maintaining the proper balance of gut bacteria and enough digestive enzymes and food preservation by facilitating the growth of desired microorganism (Caplice and Fitzgerald 1999). Each fermented food has a unique group of the microbiome and the role of microbial activity and medicinal values of the fermented food products have been documented (Chettri and Tamang 2015; Sarojnalini and Suchitra 2009). The traditional secret behind the fermentation methodologies was generally confined within the knowledge of the local communities and was handed down from generation to generation. Generally, the desirable and organoleptically pleasing characteristics were associated with the specific handling and incubation duration of raw ingredients in a precise way (Caplice and Fitzgerald 1999).

Fermented fish such as *Utonga-kupsu*, *Hentak* and *Ngari* were commonly prepared in every household for daily consumption in Northeast India. Local people have been practicing such type of food processing in the small scale

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household level for *Hentak* and *Utonga-kupsu* whereas *Ngari* is commercialized and prepared on a large scale level. These fermented fish are also known in enhancing flavor and nutritional value (Singh et al. 2015). *Utonga-kupsu* is also a fermented fish paste, which is commonly consumed by the Manipuri people located in the Cachar District of Assam North-East State of India. It is prepared by the mixture of small fish (*Esomus danricus*, *Puntius sophore*, *Amblypharyngodon mola*, *Channa punctata*, *Mystus vittatus* etc.) with *Alocasia macrorrhiza* (Hongu) as well as a little amount of mustard oil and kept in an earthen pot for 1 month. *Hentak* is a ball-like thick paste prepared by fermentation of a mixture of sun-dried fish (*Esomus danricus* and *Puntius sophore*) and petioles of Hongu as well as some small onion (Meitei Tilhou) in Manipur. The plant Hongu used in both fermented fish *Utonga-kupsu* and *Hentak* is also used as a traditional medicine in Manipur, Northeast India.

The underground stem of the plant is also used to treat Gout disease by the local people. This plant is generally prompted to allergic reaction although after fermentation with the fish no allergic reaction was reported. Both *Hentak* and *Utonga-kupsu* were specially prepared without the plant Hongu and consumed by traditional women during their pregnancy. *Ngari* is comparatively simple fermented food prepared by mixing fish (*Puntius sophore*) with salt and dried in the sun for 2–4 days, pressed gently in an earthen pot, wrapped airtight followed by storage at room temperature for 4–6 months. All the three fermented food were consumed as side-dish as well as cuisine (Eromba).

Since the fermented fish have been contributing a large portion of regular food intake and its popularities have been growing day by day in Manipur and other parts of North-east India, a proper scientific investigation is needed to identify and characterize the microbes involved in the enhancement of the nutritional value of these fermented fish. However, limited informations are available concerning the traditional procedure and scientific values of fermentation fish of Manipur, Northeast India (Thapa et al. 2004, 2006). Previous studies that have evaluated the bacterial communities in *Ngari*, *Hentak* and *Tungtap* have isolated bacterial species in the genera *Lactococcus lactis* subsp. *cremoris*, *L. plantarum*, *Enterococcus faecium*, *L. fructosus*, *L. amylophilus*, *L. coryniformis*, *L. plantarum*, *Bacillus subtilis* and *B. pumilus*, *B. cereus*, *Staphylococcus aureus* and Enterobacteriaceae population. Most of the identified strains of lactic acid bacteria characterized for future applications as probiotics (Thapa et al. 2004; Thapa 2016). But, the species were identified based on classical taxonomic methods such as phenotypic and biochemical analysis. Recent progress in molecular based phylogeny approach helps microbiologist to characterize and classify

microbe's base on their evolutionary relationships (DeLong and Pace 2001).

The present study was designed with an objective to isolate, identify and characterize the dominant bacterial species of *Utonga-kupsu*, *Hentak*, and *Ngari* using both biochemical and molecular approach. Further, we tested the Enzymatic, Antimicrobial and probiotic characteristics of the potential isolates using standard techniques.

Materials and methods

Traditional procedure for the production of *Utonga-kupsu*, *Hentak* and *Ngari*

Utonga-kupsu, a fermented fish paste, is commonly consumed by the Manipuri people in the North-East State of India. Varieties of small fish (*Esomus danricus*, *Puntius sophore*, *Amblypharyngodon mola*, *Channa punctata*, *Mystus vittatus* etc.) are selected and sun dried properly until all the water is vaporized. The dried fish are crushed into powder along with equal amount of the petioles of *Alocasia macrorrhiza* as well as a little amount of mustard oil. The mixture is kept in an earthen pot for 1 month. *Hentak* is also a fermented fish paste prepared by either or both of the two fish—*Esomus danricus* and *Puntius sophore*. The fish is sun dried completely, crushed to powder and an equal amount of the petiole of *Alocasia macrorrhiza* (Hongu) as well as some small onion (Meitei-Tilhou) is mixed together with a little amount of water. The mixture is kept in an earthen pot and is fermented for 15–20 days. *Ngari* is prepared by using only one type of fish (*Puntius sophore*), rubbed with salt, dried in the sun for 3–4 days, pressed tightly in an earthen pot, sealed airtight and then stored at room temperature for 4–6 months (Fig. 1) (Jeyaram et al. 2009).

Sample collection

Four samples of each *Utonga-kupsu*, *Hentak* and *Ngari* were collected in sterile vials from different villages—Kaptanpur (26.11°N and 91.83°E), Cachar District, Assam, Kakching Khunou, Manipur (24.40°N and 93.89°E) and Jiribam Bazar (24.80°N and 93.12°E), Manipur, respectively. The samples were stored in a cold condition with ice packs and transported to the lab within 48 h for further analysis. The four samples of each fermented fish were mixed into a single composite sample. The geographical locations of the collected samples are shown in Supplementary Fig. 1.

Fig. 1 Traditional method of preparation of **a** Utonga-Kupsu, **b** Hentak and **c** Ngari

DPPH radical-scavenging activity

Antioxidant activity of the *Utonga-kupsu*, *Hentak* and *Ngari* were determined according to the method of Majumdar et al. (2016). One gram Sample was mixed with 9 ml of 100 mM Tris–HCl buffer (pH 7.4). The mixture was properly mixed and centrifuged at $7000\times g$ for 10 min at room temperature. A known volume of supernatant was taken and added with an equal volume of DPPH solution (0.5 mM in ethanol). The mixture was left to stand for 30 min at dark. Absorbance was measured at 517 nm using a UV-spectrophotometer (Shimadzu Corporation, Japan).

Bacteriological analysis of *Utonga-kupsu*, *Hentak* and *Ngari*

One gram of each sample was diluted in 10 ml of Milli-Q water and serially diluted up to four-fold for the inoculation in Nutrient Agar (NA) and Starch Casein Agar (SCA) (Himedia). The media were poured onto the plates and different concentrations of the diluted samples were spread on the plates and incubate at 37 °C for overnight. Morphologically different colonies were selected and further subculture into freshly prepared media. Total 46 morphologically (dominant) different pure cultures were isolated and preserved in NA broth with 20% glycerol at 20 °C for further identification.

Phenotypic and genotypic characterization of isolates

The purified isolates were examined by cell morphology and by performing Gram staining, sporulation, catalase and oxidase tests according to Bergey's Manual of Determinative Bacteriology (Holt et al. 1994).

Antimicrobial activity test

Antibacterial activities of the isolated bacterial strains were tested against three pathogenic strains: *Staphylococcus aureus* (SA) (ATCC 25923), *Pseudomonas aeruginosa* (PA) (ATCC 27853) and *Escherichia coli* (EC) (ATCC 27859) using agar diffusion method in Muller-Hinton agar media according to Angmo et al. (2016).

Enzymatic assay

The isolated pure cultures were tested for the production of protease, carboxymethyl cellulase, amylase and phosphatase enzymes. Strains were streaked into Pikovskaya's agar, Starch agar, Skimmed Milk agar and Carboxymethyl Cellulose (CMC) agar incubated at 37 °C around 2–4 days. Bacteria capable of producing the respective enzyme formed a clear zone and were measured.

Evaluation of probiotic properties

Among the 46 isolates, three isolates (UKS59, HNS60 and HNS62) showing high enzymatic as well as antimicrobial

activities were further analyzed for their probiotic properties.

pH tolerance

Overnight cultures of the three isolates were inoculated into nutrient the broth. The pH was adjusted at 2.0, 4.0 and 6.0 (with 3 N and N/10 HCl). The tubes were incubated at 37° overnight. The ability of the isolates to grow in these three pH conditions was determined (Angmo et al. 2016).

Lysozyme tolerance

Tolerance to lysozyme was assessed following the method reported by Vizoso-Pinto et al. (2006).

Beta-galactosidase production

For beta-galactosidase activity, the crude bacterial liquid cultures were spread on MRS agar plates containing 60 ml X-gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) and 10 ml of IPTG (iso-propyl-thio-b-D galactopyranoside) solution as inducer (Angmo et al. 2016). The formation of blue colony indicated the *Beta-Galactosidase* production.

Antibiotic resistance

Muellar Hinton agar plate was overlaid with 100 µl of bacterial liquid culture. Antibiotic discs containing Ampicillin (10 mcg), Streptomycin (10 mcg) and Cloxacillin (10 mcg) were placed on inoculated plates under sterile conditions. After incubation for 24 h at 37 °C, the diameter (mm) of inhibition zone was measured (Angmo et al. 2016).

Biofilm production

Solid-surface-associated biofilm formation was estimated by the modified crystal violet (CV) staining method (O'Toole et al. 1999). 100 ml of each bacterial isolates were inoculated in 5 ml of freshly prepared Nutrient broth in a test tube and incubated at 37 °C overnight in shaking condition. After the incubation, the cultures were further incubated again at 37 °C for 10 h, not in the shaking condition. The cultures were stained with 1 ml of 1% CV solution for 25 min at RT, followed by washing with distilled water. The Appearance of violet color on the walls of the test tube was recorded for positive activity.

Molecular characterization of the bacterial isolates

DNA isolation

Bacterial isolates were inoculated in the liquid culture medium (NA and SCA) and incubated at 37 °C overnight at 120 rpm. The cultures were centrifuged at 5000 rpm for 5 min and the pellets were further subjected to DNA isolation (Sambrook et al. 1989). The quality of the DNA was checked by running on 0.8% agarose gel electrophoresis and the DNA was stored at – 20 °C for further analysis.

PCR of 16s rRNA gene

The 16s rRNA gene was amplified by Polymerase Chain Reaction (PCR) using primers 27-F (5'-AGAGTTT-GATGMTGGCTCAG-3') and 1492-R (5'-GGTTACCTTGTTACGACTT-3'). PCR was carried out in 25 µl total reaction volumes. The PCR reaction mixture is shown in Supplementary Table 1. The reaction condition was 94 °C for 5 min followed by 35 cycles each consisting of 40 s, denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, 3 min of extension at 72 °C. The PCR product is further purified using Gene JET purification column (K0701) and sequenced using Applied Biosystems® 3500 Series Genetic Analyzer.

Sequence analysis and phylogeny

Online similarity searches were performed using the BLAST algorithm of GenBank. Multiple sequence alignment was carried out with the CLUSTALW program. A phylogenetic tree was constructed by MEGA5 software using maximum likelihood method (Tamura et al. 2011). The taxonomic studies and properties of bacterial isolates were determined.

Results

Bacteriological analysis of *Utonga-kupsu*, *Hentak* and *Ngari*

Results show that the number of bacterial count in Nutrient agar and Starch casein agar was 14–30 and 10–18 logCFU/g, respectively. A total of 46 morphologically different bacterial strains were identified and assigned under the phylum Firmicutes (Supplementary Table 2).

Phenotypic and genotypic characterization of isolates

Phenotypic and genotypic characterizations of isolates were shown in Supplementary Table 3. All the 46 pure culture were shown positive in gram staining. 14 Isolates were catalase positive, 15 oxidase positive and 12 were positive in endospore staining.

Antioxidant activity of *Utonga-kupsu*, *Hentak* and *Ngari*

DPPH radical scavenging activity of *Utonga-kupsu*, *Hentak* and *Ngari* was determined as 40.53, 35.77 and 52.11%, respectively (Supplementary Table 2).

Antimicrobial activity test

Most of the bacterial isolates have the antimicrobial properties. Table 1 shows the list of bacterial isolates having the antimicrobial properties against the three pathogens viz SA, PA and EC. Among the isolates, HNS60 identified as *Bacillus subtilis* isolated from *Hentak* shows antimicrobial activity against to all the three pathogens and other isolates such as HNS61 shows antimicrobial activity against SA and EC. Strain HNS58, HNS47, HNS62 and UKS59 shows antimicrobial activity against PA and EC and NGS16 shows antimicrobial activity against SA and PA.

Enzymatic assay

Most of the bacterial isolated were shown good activity in the enzymatic assay. Table 2 shows the list of bacterial isolates having the enzymatic activity. Among the isolates, HNS62, HNS11, HNS12, HNS60, HNS57, UKS23, UKS59, UKS56, UKS17, UKS13, NGS44, NGS55, NGS66 were shown to have the highest activity in Pikovskaya's agar, Starch agar, Skimmed Milk agar and CMC agar.

Probiotic activity

Tolerance to acidic pH is one of the key requisites for the evaluation of probiotics. Among the three potential isolates, only HNS60 was able to grow at low pH (4.0) (Table 3). All the three tested isolates were resistant in the tested lysozyme concentration (100 mcg/ml). They also showed susceptible to the antibiotic ampicillin (10 mcg) and streptomycin (10 mcg). However the isolate HNS60 was resistant in cloxacillin (10 mcg). In the beta-galactosidase activity screening isolate, HNS60 possesses the presence of beta-galactosidase activity after 42 h

Table 1 Antimicrobial activity against human pathogens

Fermented fish	Isolate no.	SA	PA	EC
Hentak	HNS61	+	–	+
	HNS60	+	+	+
	HNS58	–	+	+
	HNS4	–	+	–
	HNS47	–	+	+
	HNS62	–	+	+
	HNS48	–	–	+
	HNS54	–	–	+
	HNS 51	–	–	+
	HNS 5	–	–	+
	HNS 12	–	–	+
	HNS 11	–	–	+
	HNS 57	–	–	+
Utonga-Kupsu	UKS 59	–	+	+
	UKS 17	–	–	+
	UKS 6	–	–	+
	UKS 56	–	–	+
	UKS 43	–	–	+
	UKS 13	–	–	+
	UKS 23	–	–	+
	NGS16	+	+	–
Ngari	NGS 55	–	–	+
	NGS 44	–	–	+

SA, *Staphylococcus aureus* ATCC 25923; PA, *Pseudomonas aeruginosa* ATCC 27853; EC, *Escherichia Coli* ATCC 27859; +, positive results; –, negative results

incubation at 30 °C. Biofilm formation was observed in UKS59 and HNS 60 isolates.

Sequence analysis and phylogeny

The 16S rRNA gene sequences from 46 bacterial isolates were deposited in the Genbank (Accession No.'s KX953112–KX953157) and subjected to BLAST in the NCBI database and the nearest representative is shown in Supplementary Table 4. Based on the sequence similarity 34 were assigned under the genus *Bacillus* and 12 under *Staphylococcus*. Within the *Bacillus*, the most dominant strain was classified as *B. licheniformis*. Other was classified as *B. aerius*, *B. altitudinis*, *B. cereus*, *B. methylotrophicus*, *B. pumilus*, *B. safensis*, *B. siamensis*, *B. sonorensis*, *B. subtilis*, *B. tequilensis*, *B. vallismortis* and *B. velezensis*. Whereas the genus *Staphylococcus* contains *S. cohnii* and *S. nepalensis* (Supplementary Table 4). Figure 2 shows the Phylogenetic

Table 2 Enzymatic activities of the isolated bacterial strains

Fermented fish	Isolate no.	Phosphatase	Amylase	Protease	Cellulase
Hentak	HNS61	+	–	+	–
	HNS62	+	+	–	+
	HNS5	+	–	+	–
	HNS4	–	–	+	–
	HNS11	–	+	+	+
	HNS12	–	+	+	+
	HNS60	–	+	+	+
	HNS47	–	+	+	–
	HNS57	–	+	+	+
	HNS93	–	–	+	+
	HNS42	–	+	–	+
	HNS48	–	+	–	+
	HNS88	–	+	–	+
Utonga-Kupsu	UKS43	+	–	+	–
	UKS6	+	–	+	–
	UKS23	–	+	+	+
	UKS59	–	+	+	+
	UKS56	–	+	+	+
	UKS17	–	+	+	+
	UKS13	–	+	+	+
	UKS34	–	–	–	+
	UKS84	–	+	–	+
	UKS74	–	+	–	–
Ngari	NGS44	–	+	+	+
	NGS55	–	+	+	+
	NGS16	–	+	–	–
	NGS46	–	+	–	–
	NGS66	+	+	–	+
	NGS67	–	+	–	+
	NGS68	–	+	–	+
	NGS85	–	+	–	+

+, positive results; –, negative results

Table 3 Probiotics characteristics of the potential bacterial isolates

Isolate no.	Growth at pH			Lysozyme	Biofilm	Antibiotics			Beta-galactosidase
	2	4	6			Ampicillin	Streptomycin	Cloxacillin	
UKS59	–	–	+	R	+	S	S	S	–
HNS60	–	+	+	R	+	S	S	R	+
HNS62	–	–	+	R	–	S	S	S	–

+, positive results; –, negative results; R, resistance; S, susceptible

relationship of the isolated strains from the fermented fish *Utonga-kupsu*, *Hentak* and *Ngari*. *B. licheniformis* from all the fermented fish samples clustered together. All the isolates clustered together according to their species relationship and exhibited a strong phylogenetic relationship. The unknown

B. species present in *Hentak* and *Ngari* were dispersed and their identity may be similar to the closest species in the phylogenetic tree.

Fig. 2 The maximum likelihood tree showing the phylogenetic position of the isolates from the traditional fermented fish

Discussion

Manipur, State of Northeast India has been known for the high consumption of the fermented fish products. Traditional people of Manipur believe that consumption of these fermented fish helps in digestion and cures allergic reaction that is caused by the consumption of local vegetables, although there is no proper investigation regarding its scientific value in respect of its bacterial communities. The present study deals with the identification of bacterial isolates from fermented fish products and includes the study of their antimicrobial as well as their enzymatic activities. In any fermented food, diversity of microorganism is present which has the capacity of producing many useful enzymes viz-oxidase, β -galactosidase, amylase, aesculin hydrolysis, starch hydrolysis, nitrate to nitrite reduction as well as antibiotics and other compounds which are useful in bioremediation (Majumdar and Basu 2010).

In the present study, *Bacillus* was predominantly found in *Utonga-kupsu*, *Hentak* and *Ngari*. These species were also reported from other Asian fermented fish products such as *Namplaa* and *Kapi*, fermented fish products of Thailand (Tanasupawat et al. 1992). The dominance of *Bacillus* in the present study due to their ability to form endospores to survive under the unfavorable conditions (Crisan and Sands 1975). The survival of *Bacillus* during anaerobic fermentation can be explained by their ability to utilize nitrate as an alternative electron acceptor by reducing nitrate to nitrite using respiratory nitrate reductase (NarGHI) and further reduced to ammonia using nitrite reductase (NasDE) (Ramos et al. 2000). In the present study, seven bacterial isolates were classified under the genus *Staphylococcus cohnii* and five under *Staphylococcus nepalensis*. *Staphylococcus cohnii* strain has been identified previously during traditionally fermented sausages in Southern Greece (Drosinos et al. 2007). They are also known to produce a large amount of industrially important enzymes. The Majority of the species under the genus *Staphylococcus* is harmless and present as a commensal on the skin and mucous membranes of many organisms, including humans. Coagulase-negative staphylococci (CNS) were found as natural flora of fermented meat products in relatively high numbers (Miralles et al. 1996; Casaburi et al. 2005).

The possible antioxidant activity of the three fermented food *Utonga-kupsu*, *Hentak* and *Ngari* might come from the breakdown of protein which leads to the release of the peptides during fermentation (Phadke et al. 2014). Studies indicate that the antioxidant properties of *ngari* depend on protein concentration and period of fermentation. The same study also indicates that the traditional fermented fish product (*Ngari*) could be used in alleviating hypertension

and lipid oxidation (Phadke et al. 2014). In the present study, the radical scavenging activity of the three fermented fish was determined to be in the range 35–52 (Supplementary Table 2) which was slightly lower than *Shidal*, a traditional fermented fish of Northeast India. Usually, peptides from fermented fish products have been reported to act as antioxidants (Majumdar et al. 2016). Salt-fermented food of Philippine's showed 24.3–61.5% DPPH-scavenging activity in 80% ethanolic extract (Peralta et al. 2008).

Most of the isolated strains showed high amylase, protease, and cellulase activities and thereby can improve the quantity, availability, and digestibility of dietary nutrients in the body. The production of various enzymes and vitamins by the genus *Bacillus* has been proved by the earlier study (Parvez et al. 2006). Present study identified strains showed antimicrobial activity against the bacterial pathogens and thus can lower the unwanted microorganisms in the fish fermented product, ultimately aiding in the preservation process (Einarsson and Lauzon 1995). The antimicrobial compounds produced by the genus *Bacillus* and *Staphylococcus* can be used for combating the growth of infectious microorganisms in the fermented fish as a purified form or as a crude culture (Liasi et al. 2009).

The present study also evaluates the probiotics characteristics of the three potential isolates, as they are safe and cause various health benefits. The most important criteria for probiotic bacteria are to survive and colonize inside the human gastrointestinal tract (GIT). The in vitro study on pH tolerance revealed that isolate no. HNS60 can tolerate acidic condition and can even survive at pH 4. Acid tolerance of bacteria is important not only for with standing gastric stress, but it also enables the strain to survive for a longer period in high acid carrier foods (Angmo et al. 2016). The strain HNS60 also showed lysozyme tolerance activity (mgc/ml) as well as antimicrobial activity against SA, PA and *E. coli*. Lysozyme can damage the bacterial cell walls by catalyzing the hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan. In the present study, all the three tested isolates were shown resistant in the lysozyme assay and thereby allowing them to colonize on the mucosal surface (Maga et al. 2012).

Biofilm formation is an important factor for colonizing themselves in the large intestine and can directly involve in the breakdown of complex insoluble polymeric substances. This also allows an added advantage for competing for nutrients with non-adherent organisms (Macfarlane and Macfarlane 2006). β -galactosidase is a key component for the energy production and acts as a carbon source. It also helps people having lactose intolerance by converting lactose to galactose and glucose leads to proper digestion of lactose containing products (Salehi et al. 2009).

The allergic reaction by *Alocasia macrorrhiza* comes from the calcium oxalate crystals, causing injury in internal human mouth and throat tissue. The underground stem is edible and is only safe to eat after prolonged cooking, which breaks down the irritating crystals of calcium oxalate (Krauss 1993). During sun drying, the calcium oxalate converts to calcium carbonate which might be the reason for preventing the gout attacks by neutralizing acids in the body (uric acid) (Holmes-Farley and Burke 2002). This plant property along with the microbial fermentation adds value to the medicinal property of the food in terms of their probiotic and antioxidant activities.

This is the first scientific investigation concerning the molecular identification of bacterial isolates present in *Utonga-kupsu*, *Hentak* and *Ngari* from Manipur, North-East India. Analysis revealed the presence of *Bacillus* and *Staphylococcus* species with a high degree of antimicrobial and enzymatic activities in all the three fermented fish products. The isolate HNS60 possesses antimicrobial, antioxidant as well as probiotics activities. Further scientific study with the whole genome sequence of the bacterial isolates will reveal their functional potential for industrial and biotechnological applications.

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