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Microbiology of *khorisa*, its proximate composition and probiotic potential of lactic acid bacteria present in *Khorisa*, a traditional fermented Bamboo shoot product of Assam

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Khorisa is an ethnic fermented tender bamboo shoot product prepared in Assam. It is relished in a variety of forms and food preparation including fish and meat. However, the study on the microbial dynamics and nutritional value of *khorisa* is scarce. In this present report, we have isolated, identified, and characterized the predominant lactic acid bacteria (LAB) from three different varieties of *Khorisa.* Their functional properties and antimicrobial effects against foodborne pathogen have been studied. Microbiological analysis showed the presence of 5 different strains of LAB (*Lactobacillus plantarum*, *L. brevis, L. paracasei* subspecies *paracasei, L. pentosus*, and *L. collinoides* ranging upto 10^7cfu/mL. Bacteriocin was partially purified to test sensitivity against enzymes, temperature, and pH. The fresh and fermented bamboo shoots were evaluated and compared for their different nutritional parameters. Cyanide content before and after fermentation has also been studied. The present study emphasizes the functional properties of the lactic acid bacteria present in *khorisa*, which makes it one of most suited traditional food with immense health benefits

Keywords: *Khorisa*, Lactic acid bacteria, Bacteriocin
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Introduction

Bamboo shoots are being used as a food supplement for decades in the human diet, yet the food potential of fermented bamboo shoots remained little known all the years. Bamboo shoot is a part of the traditional Asian medicine, especially in China and Japan. It has great medicinal properties as mentioned in Ayurveda. It acts as a source of antioxidant, anti-ageing, anticancer, and prevents cardiovascular diseases. The presence of flavonoids and glycosides imparts it the antimicrobial activity[1–3]. Lactic acid bacteria (LAB) are naturally present in most living plants and spontaneous lactic acid fermentation occurs when vegetables are stored in anaerobic condition[4,5]. Since food safety and nutrition is a major health concern, application of antimicrobial peptides from lactic acid bacteria that inhibit food pathogens without any toxic or other adverse effects has received considerable attention. The bacteriocin from lactic acid bacteria was first reported by Gratia[6] in 1925 and thereafter, bacteriocin production has been reported in many species of bacteria attracting considerable interest in terms of food safety[7]. Further, bacteriocins are food-grade, which enables food scientists to develop desirable flora in the fermented foods or prevent the development of specific unwanted (spoilage and pathogenic) bacteria in both fermented and non-fermented foods by using broad and narrow host range bacteriocin. *Khorisa* is an ethnic traditional fermented bamboo shoot product prepared by the Assamese people. Despite the use of this fermented bamboo shoot product as food, the nutritional content of this edible food is not well characterized. The present study aims to isolate and identify the predominant lactic acid bacteria from *Khorisa* and to employ bacteriocin from LAB to enhance the stability of different food products.

Materials and Methods

Collection of samples

Samples of young succulent bamboo shoots namely *Dendrocalamus hamiltonni* (*Kako*), *D. giganteus* (*Jati*) and *Bambusa balcooa* (*Bhuluka*) (Plate 1a) were collected from the bamboo field of the Department of Agricultural Biotechnology, AAU, Assam. In the

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Supplementary tables are available online only

traditional method of *khorisa* preparation, usually bamboo shoots are defoliated and pound in a *dheki* (leg operated pounder). These are then tightly packed in earthenware pots or *chunga* (hollow bamboo stem), covered with banana leaves, and allowed to ferment in natural anaerobic condition for 7-15 days. Usually, completion of the fermentation is indicated by the typical *Khorisa* flavor, smell, and taste. In the lab, the outer sheath of the young bamboo shoots was removed and washed thoroughly with water. The tender shoots were then grated with the help of a grater. The grated material was filled to the brim with the glass jars and allowed to ferment for 7-12 days (Plate 1b).

Determination of pH and acidity

Samples (10 g) of each product were mixed with 90 mL of 0.85 % (w/v) sterile physiological saline and homogenized in a Stomacher lab blender (Seward, UK) for one minute. The pH of the samples was measured every day for 10 days using a digital pH meter (Type 361, Systronics). Titratable acidity was calculated by titrating the filtrate of the homogenate with 0.1 N NaOH to an endpoint of phenolphthalein (0.1 % w/v in 95 % ethanol)[8].

Microbiological analysis of *khorisa*

Samples (10 g) of each product were mixed with 90 mL of 0.85 % (w/v) sterile physiological saline and homogenized in a Stomacher lab blender (Seward, UK) for 1 min. A serial dilution in the same diluents was made. Lactic acid bacteria were enumerated on de Man Rogosa and Sharpe (MRS) agar plates and incubated at 30 °C for 48-72 h in an anaerobic gas pack system. *Enterobacteria* were isolated on Enterococcus Confirmatory Agar, total viable counts were determined using Plate Count Agar and incubated at 30 °C for 48 h. Yeast and molds were isolated on Potato Dextrose Agar. The purity of the LAB isolates was checked by streaking again and sub-culturing on fresh MRS agar.The colonies were randomly picked from selected plates (having 50-100 colonies per plate) to obtain representative strains at regular intervals of fermentation time and the isolates were purified by successive sub-culturing in the corresponding broth and streaked onto the agar surface. After microscopic examination, purified cultures were grown on slants of the same medium and stored at 4 °C.

Plate 1-a) — Different varieties of Bamboo shoot, *Kako* (*Dendrocalamus hamiltonni*), *Jati* (*Dendrocalamus giganteus*), and *Bhuluka* (*Bambusa balcooa*) and b) Lab made fermented *khorisa.*

Characterization of the bacterial strains

All strains were initially subjected to Gram staining[9]. Cell morphology and motility of the isolates were checked using a phase-contrast microscope (Olympus CH3-BH-PC, Japan). Catalase production was tested by placing a drop of 10 % H_2O_2 solution on the isolates. Gas production from glucose and growth in the presence of different concentration of NaCl were carried out and arginine hydrolysis tests were done. The APILAB PLUS database identification software (Biomeriux, France) was used to identify the strains. Sugar fermentation pattern of LAB strains was determined using API 50 CH Rapid fermentation strip (API, France) in the medium following manufacturer's instruction.

Nutritional analysis of fresh bamboo shoot and *Khorisa* samples

The pH and titrable acidity of the samples were measured regularly for 10 days using a digital pH meter (Type 361, systronics) calibrated with standard buffer solution. Total soluble solids (TSS) were determined by a digital band refractometer (0.53 %) and were expressed in Brix[10]. The sugar was determined by Anthrone method[11]. The amount of crude protein was estimated by Lowry's method and iron[12]. The calcium and phosphorus content was determined by flame photometry using a systronic flame photometer (Model MM III). The amount of calcium was calculated from samples reading after plotting it on a standard curve obtained from $CaCO_3$. Crude fiber was estimated by following the protocol as described in AOAC[13].

Total cyanide estimation

Total cyanide content was determined by alkaline picrate spectrophotometric method[14]. The cyanide content was determined in the tip, middle, and base section of the bamboo shoot and also in *khorisa* (Supplementary table 1).

Enzymatic profile

The enzymatic profile of the isolated lactic acid bacteria were assayed using API-zym (API, Biomeriux, France) galleries by testing for the activity of the following 19 enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl glucosamine, α-mannosidase, and α-fucosidase.

Extraction and purification of bacteriocin

The extraction and purification were done by the methods given by Mourad *et al.*[15] and Gautam & Sharma[16] with some modification. The strains were inoculated in MRS broth media for 48 h at 30 °C and were centrifuged at 8000 rpm for 30 minutes at 4 °C. The supernatant was then filtered through a filter paper. This solution was considered as the crude extract.

Bacteriocin was partially purified by salt saturation method. The crude extract was saturated with 70 % ammonium sulfate and stirred with the help of magnetic stirrer for 24 h to precipitate out the proteins. Then, centrifugation was done at 8000 rpm at 4 °C for 1 h and pellet was resuspended in 25 mL of 0.1 M potassium phosphate buffer pH 7.0. To this solution, 5 % equivalent of TCA was added. The mixture was centrifuged at 8000 rpm for 20 minutes, after which the supernatant was decanted. The pellet was dissolved in phosphate buffer and centrifuged at 8000 rpm for 10 minutes at 4 °C. The pellet containing bacteriocin was washed with water and centrifuged again at 8000 rpm for 25 minutes at 4 °C. Purified bacteriocin activity was confirmed by well diffusion assay. No inhibition zone indicates that the bacteriocin activity was retained in the pellet.

Bacteriocin assay of the efficient LAB strains

Isolates of LAB from *khorisa* were examined for their antagonistic activity by agar well diffusion method (AWDA) following the protocol[17] with some modification. The LAB strains were screened against indicator organisms *Staphylococcus aureus* (ATTC2346), *Listeria monocytogenes* (ATCC3452), *L. innocua* (ATCC3154), and *Enterococcus cloacae* (ATCC 35030).

Characterization of the purified bacteriocin

Characterization was done by following the methods given by Gautam & Sharma[16] of the following parameters.

Heat resistance

The sensitivity of the purified bacteriocin was assessed by adding 0.5 mL of the purified bacteriocin to 4.5 mL of sterile Nutrient broth in test tubes, plugging with cotton and aluminum foil, and keeping in an autoclave at 121 °C for 15 minutes. It was further tested for antimicrobial activity by the well diffusion method.

pH sensitivity

The effect of pH on bacteriocin activity was checked by pH of the tubes containing 4.5 mL of nutrient broth to different pH ranges from 4.5-6.0, keeping for one hour at room temperature, and further tested for antimicrobial activity.

Sensitivity to proteolytic enzymes

The sensitive nature of bacteriocin to proteolytic enzymes such as papain, proteinase K, chymotrypsin, and trypsin was tested in test tubes containing 0.15 mL of phosphate buffer (0.5 M, pH 7.0), 0.15 mL of the bacteriocin, and 0.15 mL of trypsin, chymotrypsin, papain, proteinase K (0.25 mg/mL) were added. This is considered as the enzyme reaction tube. Control was taken to show that that the inhibition was not caused by the phosphate buffer; control tube contained 0.3 mL of phosphate buffer devoid of bacteriocin and enzyme and another containing the 0.15 mL phosphate buffer.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA), followed by Duncan's Multiple Range Test[18].

Results and Discussion

Microbiology of the fermented bamboo shoot

Microbiology of *khorisa* depicts the presence of enterobacteria, aerobic mesophiles, and yeast loads (Table 1 and Plate 2). LAB has been demonstrated to be the predominant microorganism involved in the fermentation of bamboo shoot. LAB appeared from

Table 1 — Changes in the microbial load during *Khorisa* fermentation

Microbes	Fermentation time (Days) cfu/mL							
	0	1	2	3	4	5	6	7
Enterobacteria	$0.9x10^5$	$0.8x10^4$	$0.7x10^4$	$0.5x10^4$	$0.3x10^3$	-	-	-
Aerobic mesophiles	$9.5x10^5$	$6.7x10^3$	$5.1x10^3$	$4.9x10^2$	$4.5x10^2$	-	-	-
Yeast	$2.1x10^3$	$1.5x10^2$	$0.5x10^2$	-	-	-	-	-
LAB	-	-	$3.9x10^4$	4.6x105	$5.1x10^6$	$7.3x10^6$	$7.5x10^7$	$7.5x10^7$
Molds	ND							

*Data represents the means of 3 samples, ND-Not Detected

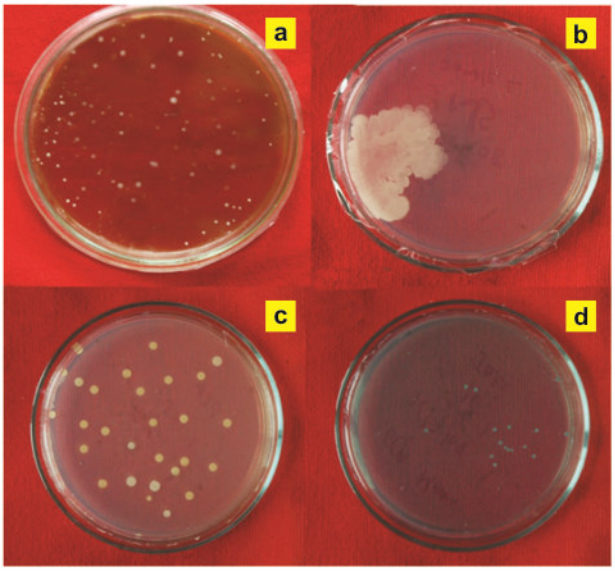

Plate 2 — Colony morphology of different microbes in different selective media. a) Lactic acid bacteria on MRS media, b) Yeast on YEA media, c) Aerobic mesophilles on PCA media, and d) Enterobacteria on ECA media.

the second day of fermentation and ranged from 3.9 x 10^4 to $7.5x10^7$ cfu/mL on day 7. The presence of high number of LAB indicates the formation of lactic acid during fermentation which inhibited the growth of other microorganisms. All the LAB isolates were found to be Gram-positive, heterofermentative rods (Plate 3), catalase negative, non-motile, released gas from glucose, ammonia from arginine (Table 2). A total of five, each in duplicate, isolates were considered for identification and characterization. The strains KA1/BA1 were identified as *L. plantarum*, JA1/BA3 as *L. brevis*, KA3/BA2 as *L. paracasei*, KA4/JA2 as *L. pentosus* and KA2/JA3 as *L. collinoides*. Similar results were shown in *Mesu, Soidon, Soibum*, traditional fermented bamboo shoot products of Sikkim, Darjeeling Hills and Manipur. The number of LAB, yeast, and aerobic mesophiles were found to be in the range of 10^2, 10, and 10^2 cfu/g, respectively. Molds were not detected. In another study, the predominant functional lactic acid bacteria were reported to be *Lactobacillus brevis*, *L. curvatus*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides* subsp. *mesenteriodes, L. fallax, L. lactis, L. citreum*, and *Enterococcus durans*[19]. In *soibum*, traditional fermented bamboo shoot product of Manipur, *Bacillus* and *Micrococcus* species have been reported[20]. Fermented bamboo shoot product called *Naw-mai-dong* or *Nor mai-dorng* of Thailand have also been reported to contain *Lactobacilli*, *Leuconostoc*, and *Pediococci*[21,22].

Functional properties of LAB

The representative strains were randomly selected from each grouped strains having similar morphology. A total of seven strains of LAB were consolidated and later identified on the basis of sugar fermentation pattern using API kit, France, BioMerieux (Table 2). The LAB strains identified as *L. plantarum*, *L. collinoides*, *L. paracasei* subsp. *paracasei*, *L. pentosus*, and *L. collinoides*. All the identified strains grew well in the temperature range of 25 to 45°C, pH range of 2.5-6 and 4 and 6.5 % concentration of NaCl, but were unable to grow in 10 % NaCl, which suggest that these strains are intolerant to high salt (Supplementary table 2). In *Mesu*, *Soibum*, *Soidon*, and *Soijim*, LAB were phenotypically characterized on the basis of cell morphology, growth at different temperature (10, 15, 45 °C) and in various concentration of salt. It was found that lactic acid bacteria were heterofermentative rods unable to grow at 45 °C[19].

Influence of fermentation on nutritional parameters

Bamboo shoots are rich sources of several nutritional components like protein, carbohydrates, vitamins, minerals, fiber, phytosterol, phenol, and less fat[23]. The present research studied and compared the nutrient composition of fresh and fermented bamboo shoot. The study showed that fermentation not only improves the flavor, texture, and appearance, but also

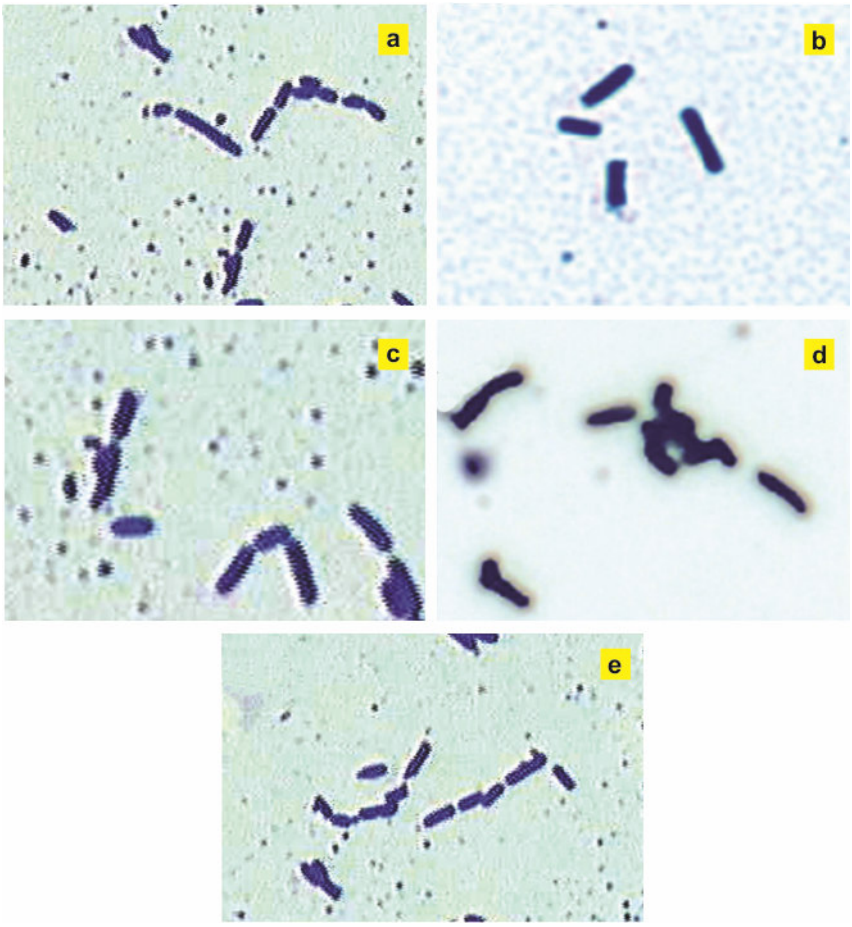

Plate 3 — Gram staining of different species of Lactic acid bacteria. a) *Lactobacillus brevis*., b) *Lactobacillus plantarum*., c) *Lactobacillus paracasei* subsp. *Paracasei*., d) *Lactobacillus pentosus*., and e) *Lactobacillus collinoides.*

Table 2 — Carbohydrate fermentation pattern of the LAB isolated from *Khorisa*

Carbohydrate	Strain designation				
	JA1/BA3 Lactobacillus brevis	*KA1/BA1 Lactobacillus plantarum*	*KA3/BA2 Lactobacillus paracasei* subsp. *paracasei*	*KA4/JA2 Lactobacillus pentosus*	*KA2/JA3 Lactobacillus collinoides*
Glycerol	-	-	-	-	-
Erythritol	-	-	-	-	-
D Arabinose	+	+	-	-	-
L. Arabinose	-	-	+	+	+
Ribose	+	-	+	-	+
D-xylose	+	-	-	-	+
L-xylose	-	-	-	-	-
Adonitol	-	-	-	-	-
B-methyl D-xylose	+	-	-	-	+
Galactose	+	+	+	+	+
D-glucose	+	+	+	+	+
D-fructose	+	+	+	+	+
D-mannose	-	-	+	+	+
L sorbose	-	-	-	-	-
Rhamnose	-	+*	-	+	-

(*Contd.*)

Table 2 — Carbohydrate fermentation pattern of the LAB isolated from *Khorisa* —*(Contd.)*

Carbohydrate	Strain designation				
	JA1/BA3 Lactobacillus brevis	*KA1/BA1 Lactobacillus plantarum*	*KA3/BA2 Lactobacillus paracasei* subsp. *paracasei*	*KA4/JA2 Lactobacillus pentosus*	*KA2/JA3 Lactobacillus collinoides*
Mannitol	-	+	+	+	+
Sorbitol	-	+	-	+	+
α-Methyl D mannoside	+	-	+	-	+
α-Methyl D-glucose	+	+	+	-	+
N-acetyl glucosamine	-	+	-	+	+
Amygdalin	-	+	+	+	+
Arbutin	-	+	-	+	+
Esculin	-	+	+	+	+
Salicin	+	-	-	+	+
Cellobiose	-	+	+	+	+
Maltose	-	+	-	-	+
Lactose	+	+	+	+	+
Melibiose	+	-	+	+	+
Sucrose	-	+	+	+	+
Trehalose	-	-	+	+	+
Inulin	-	+	+	-	+
Melezitose	-	+*	+	+	-
D-raffinose	-	+	+	-	-
Starch	-	-	-	+	+
Glycogen	-	-	+	-	+
Xylitol	-	-	-	-	-
β-gentiobiose	-	-	-	+	-
D-Turnose	-	-	-	+	-
D-Lyxose	+	+	-	+	-
D-Tagatose	-	-	-	-	-
D-Fucose	-	-	+	-	+
L-Fucose	-	-	-	-	-
D-Arabitol	-	-	-	-	+
D-Arabitol	-	-	-	+	-
Gluconate	-	+	-	-	-
2-keto gluconate	-	-	-	-	-
5-keto gluconate	-	-	-	-	-

helps to enrich the nutritional value (Table 3 and 4). Enhancement of nutritive value in *soibum* has been reported earlier[24].

Change in pH, TSS, and titratable acidity

During the course of fermentation, decrease in pH was observed. TSS and titrable acidity were found to increase during fermentation as compared to the fresh samples. Titrable acidity was measured in % lactic acid**.** The low pH makes organic acid liposoluble, allowing them to break through the cell membrane and enter the cytoplasm of pathogen[25]. Iron content increased, whereas no change was found in calcium and phosphorus content in all *khorisa* samples. Earlier, increase in lactic acid content from 0.04 to 0.95 % and decrease in pH from 6.4 to 3.8 during fermentation period of 10 days in *Mesu* (fermented bamboo shoot product of Sikkim) has been reported[26]. High acidity and consequently low pH observed was likely due to the utilization of free sugar by LAB and yeasts[27,28].

Change in sugar, protein, crude fiber, ascorbic acid, and alkaloid

Sugar content in all the three varieties of bamboo shoot decreased during fermentation. The lower sugar content indicates that the most of the sugar may have been used up during fermentation, which was ultimately converted into lactic acid[29]. There was no significant change in protein content in fresh shoot and *Khorisa*, but varied in the various species from different origin, ranging from 1.8 to 25.8 % (dry weight basis). Crude fiber was found to decrease significantly in all the samples of *Khorisa*. However, crude fiber content for different species was reported to be in the range of

23.1-35.5 %. The ascorbic acid content has also been found to increase in fermented bamboo shoot product and the range varied from 2.55-4.97 mg/100 g. An earlier study reported ascorbic acid content in the range of 3.0-12.9 mg/100g in fresh edible bamboo shoot[30]. It has been reported that during fermentation of *soibum,* degradation of protein and increase in free amino acid was observed[31,32]. There was a significant decrease in the crude fiber content after fermentation. The crude fiber content was in a range between 12.14-14.99 % in the fresh bamboo shoot, which decreased to a range of 8.96-11.13 % in fermented *Khorisa*. However, the crude fiber content for different species was reported to be in the range of 23.1-35.5 %[30]. The decrease in crude fiber content may be due to the breakdown of cellulose and lignin to simple sugar.

Mineral content

No significant difference was observed in the mineral composition of the fresh and fermented bamboo shoot product. Both, the fresh bamboo shoots and *khorisa* contained calcium, Iron, and phosphorus. However, in an earlier study, an increase in iron and magnesium level in *Lung-siej* (fermented bamboo shoot product of Meghalaya) compared to raw bamboo shoot has been reported[33].

Cyanide content

Bamboo shoots contain the cyanogenic glycoside taxiphyllin, which is a p-hydroxylated mandelonitrile tiglochinin. Taxiphyllin is further hydrolysed to glucose and hydroxybenzaldehyde cyanohydrin. Benzaldehyde cyanohydrin then decomposes to hydroxybenzaldehyde and hydrogen cyanide. It is a potent metabolic poison, which may cause chronic and acute toxicity. According to a WHO report, concentration of cyanide in the immature shoot tip of bamboo is 8000 mg/kg of hydrogen cyanide[34], whereas another study reported that bamboo shoots contain as much as 1000 mg/kg of hydrogen cyanide in the apical part[35]. A sample of *Dendrocalamus giganteus* contained, on average, 894 mg/kg of hydrogen cyanide[36]. it is known to inhibit many other enzymes in an animal system.

In the present study, results show that the tip section of the bamboo shoot contain the highest amount of cyanide, which decreases towards the base. The cyanide content was found to be highest (381.4 ppm) in the tip section of *Jati* and lowest (88.96 ppm) in *Bhuluka*. In the middle section, cyanide content was highest (87.8 ppm) in *Jati* and lowest (66.66 ppm) in *Kako*. In the base section, cyanide content was highest in (53.86 ppm) in *Jati* and lowest (43.93 ppm) in *Bhuluka*. During the period of fermentation, it was observed that cyanide content decreased in all varieties of bamboo shoot (Table 5). It is evident from the reviews that bamboo shoots contain cyanogenic glycoside, which is toxic for human health[37]. So, safe consumption method must be followed without disturbing the nutrient reserve. In this paper, we have reported that during fermentation cyanide level reduces significantly. Thus, fermentation acts as a detoxification process and can be safely used as an efficient food processing method.

Table 3 — Nutritional evaluation of *Khorisa* sample

Parameters	Varieties			CD (0.05)
	Jati	*Kako*	*Bhuluka*	
pH	3.05[a]	3.01[a]	3.01[a]	0.1003
TSS (°Brix)	7.73[a]	6.74[b]	4.78[c]	0.0220
Titrable acidity % as lactic acid	2.283[c]	2.800[a]	2.740[b]	0.040
Sugar (mg/100g)	1.743[c]	2.66[b]	1.85[a]	0.183
Iron (mg/100 g)	2.303[a]	2.358[a]	1.715[b]	0.2845
Calcium (mg/100 g)	1490.500[c]	1689.25[b]	2007.25[a]	13.087
Ascorbic acid (mg/100 g)	2.553[c]	4.978[a]	3.835[b]	0.008
Phosphorus (mg/100 g)	10.408[b]	15.988[a]	10.748[b]	0.651
Total alkaloid (%)	0.23[c]	0.28[b]	0.29[a]	0.024
Protein (g/100g)	1.42[b]	1.58[c]	1.23[a]	0.036
Crude fibre (%)	8.965[c]	11.138[a]	9.90[b]	0.407

* In column, means followed by the same letter are not significantly different among themselves at p=0.05

Table 4 — Nutritional evaluation of fresh Bamboo shoots

	Varieties			CD (0.05)
	Jati	*Kako*	*Bhuluka*	
pH	5.5[a]	5.03[b]	5.57[a]	0.1003
TSS (°Brix)	5.803[b]	4.26[c]	6.703[a]	0.0220
Titrable acidity % as lactic acid	0.473[a]	0.432[b]	0.323[c]	0.040
Sugar (mg/100g)	3.06[a]	2.51[b]	2.73[b]	0.183
Iron (mg/100 g)	2.103[a]	2.090[a]	1.685[b]	0.132
Calcium (mg/100 g)	1494.25[c]	1605.25[b]	1988.00[a]	37.077
Ascorbic acid (mg/100 g)	2.553[c]	4.978[a]	3.835[b]	0.008
Phosphorus (mg/100 g)	10.338[b]	15.913[a]	9.023[c]	0.257
Total alkaloid (%)	0.23[c]	0.28[b]	0.29[a]	0.024
Protein (g/100g)	1.42[b]	1.58[c]	1.23[a]	0.036
Crude fibre (%)	8.965[c]	11.138[a]	9.90[b]	0.407

*In column, means followed by the same letter are not significantly different among themselves at p=0.05

Identification and biochemical characterization of the LAB strains

All the strains isolated from three different samples were identified on the basis of carbohydrate fermentation pattern. The biochemical test of LAB strains was performed based on colorimetric identification, utilizing 49-carbohydrate test. It was found that all the isolates showed lactose, D-glucose, and D-fructose utilization.

Except for *L. plantarum*, other strains identified showed cellobiose utilization. However, none of them were found to utilize glycerol and erythritol. Further, the enzymatic activities of the strains were also assayed (Table 6) using API-zym kit. It is of relevance for selection of strains as potential starter cultures based on superior enzyme profile, especially peptidases and esterases for acceleration, maturation, and flavor development of fermented product[38]. The strains showed relatively moderate esterase (C4) and strong arylamidase activities. The absence of proteinase and the presence of high peptidase (leucine arylamidase, valine arylamidase, and cystine arylamidase) and esterase lipase activities produced by LAB isolated from the *Khorisa* indicates their potential use in the production of typical flavor and also the possibility of being used as a starter culture.

Table 5 — Cyanide content in ppm (mg HCN equivalent / kg bamboo shoot)

Days of fermentation	Cyanide content (ppm) Varieties		
	Jati	*Bhuluka*	*Kako*
0	268.1[a]	86.06[a]	122.95[a]
1	87.8[b]	66.93[b]	66.66[b]
2	53.86[c]	53.93[c]	53.53[c]
3	43.61[d]	49.30[d]	42.80[d]
4	30.70[e]	38.60[e]	34.03[e]
5	26.05[f]	32.43[f]	27.10[f]
6	18.30[g]	22.90[g]	21.03[g]
7	14.10[h]	16.30[h]	16.50[h]
CD (0.05)	0.006	0.054	0.085

In column, means followed by the same letter are not significantly different among themselves at p=0.05

Bacteriocin assay of LAB isolates

All the isolates showed a clear zone of inhibition against the indicator organism (Plate 4 and Supplementary table 3). Maximum zone of inhibition was produced by *L. plantarum* against *L. monocytogenes* and followed by *L. brevis* against *L. innocua.* Previous study has also reported one LAB strain NI-33, isolated from the edible fermented bamboo shoot as a potential producer of bacteriocin, which was effective in controlling the growth of food spoilage microorganism[39]. Likewise, the inhibitory action of bacteriocin by *L. plantarum* IB2 (BFE 948) against *Staphylococcus aureus* isolated from *Inziangsang* has been reported[40].

Sensitivity of crude bacteriocin to temperature, pH, and enzymes

The bacteriocin produced by all the strains retained its activity even after treatment at 121 ºC for 15 minutes, a property that is typical for acteriocins.

Table 6 — Enzymatic profile using API zym system of LAB isolated for *Khorisa*

Enzymes	LAB strains (Activity in nanomoles)				
	Lactobacillus brevis	*Lactobacillus plantarum*	*Lactobacillus paracasei* subs. *paracasei*	*Lactobacillus pentosus*	*Lactobacillus collinoides*
Control(w/o enzyme)	0	0	0	0	0
Phosphatase alkaline	0	0	0	0	5
Esterase (C4)	10	5	10	0	10
Esterase lipase (C8)	10	10	5	10	0
Lipase (C14)	5	5	5	0	5
Leucine arylamidase	≥40	≥40	≥40	≥40	≥40
Valine arylamidase	>40	>40	>40	≥40	20
Cystine arylamidase	≥40	≥40	≥40	10	5
Trypsin	0	0	0	0	0
α-chymotrypsin	0	0	0	0	0
Acid phosphatase	≥40	10	10	10	0
Nephtol-AS-BI phospholydrolase	5	5	10	0	0
α-galactosidase	0	10	0	0	5
β-galactosidase	10	10	10	10	10
β-glucuronidase	20	20	20	20	10
α-glucosidase	10	≥40	10	≥40	10
β-glucosidase	20	20	10	20	10
N-acetyl-β-glucosamine	5	0	10	10	0
α-Mannosidase	0	0	0	0	0
α-fucosidase	0	0	0	0	0

*Data represents the means ± SD of 3 replicates
*0 no enzyme activity 5, 10, 20, 30, > 40 indicates nanomoles of hydrolyzed substrate after 6 h of incubation at 30 ºC.

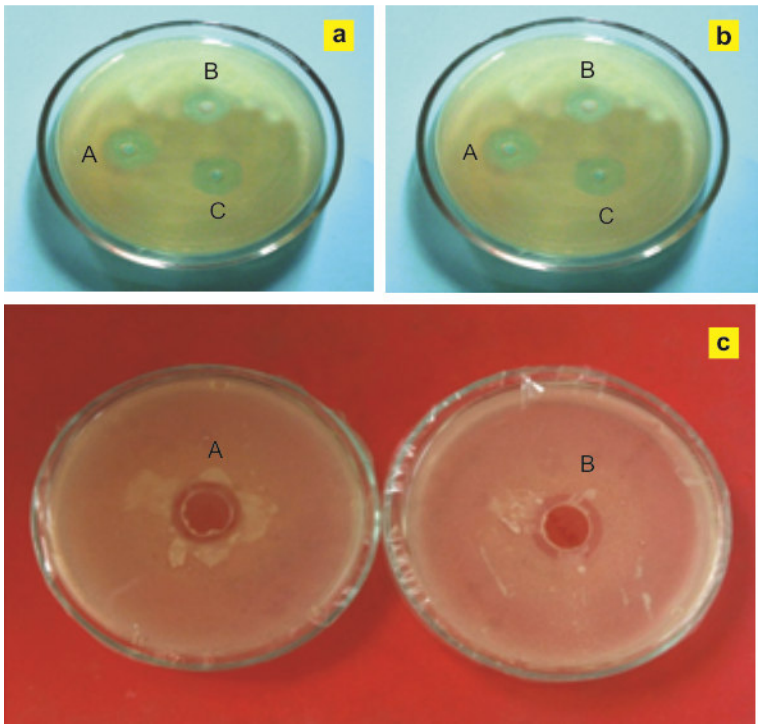

Plate 4 — Inhibition zone produced by LAB against a) *Staphylococcus aureus* CONTROL (well A) *Lactobacillus paracasei* subsp *paracasei* (well B), *Lactobacillus collinoides* (well C), b) *Listeria monocytogenes* CONTROL (well A), *Lactobacillus plantarum* (well B), *Lactobacillus pentosus* (well C), *Lactobacillus brevis* (well D), and c) *LISTERIA* CONTROL (well A), after treatment at 121°C for 15 min (well B).

Table 7 — Effect of heat treatment, pH and proteolytic enzyme on the bacteriocin produced by the LAB isolated from *Khorisa*

Treatment	Bacteriocin-producing strain				
	Lactobacillus brevis	*Lactobacillus plantarum*	*Lactobacillus paracasei* subsp. *paracasei*	*Lactobacillus pentosus*	*Lactobacillus collinoides*
Heat treatment					
121°C/15 min	+	+	+	+	+
pH					
4.0	+	+	+	+	+
4.5	+	+	+	+	+
5.0	+	+	+	+	+
6.0	+	+	+	+	+
Enzymes					
Proteinase K	-	-	-	-	-
chymotrypsin	-	-	-	-	-
Trypsin	-	-	-	-	-
Papain	Partially active	Partially active	Partially active	Partially active	Partially active

Keys: + = Resistant, no change in bacteriocin activity
- = Sensitive, inhibition of bacteriocin activity

This was inconsistent with the previous studies[41–43]. In addition, stability of bacteriocin even after treatment at 121 °C for 20 minutes has been reported earlier[44]. LAB were found to be active in the pH range of 4-6. Bacteriocin activity from other fermented product has also been reported in the pH range of 6·0–9·0[45]. The bacteriocin was treated with proteinase K, trypsin, chymotrypsin, and papain. The results showed that the bacteriocin activity was completely lost when treated with Proteinase K, trypsin, and chymotrypsin, but retained its activity when treated with Papain. This observation suggests that the bacteriocin produced by the strains under study were proteinaceous in nature (Table 7). Bacteriocins possess a protein moiety,

which is responsible for the inhibition of the target organisms. Many bacteriocins produced by LAB act against bacteria closely related to the producer organisms and also inhibit *Listeria.* The sensitivity of bacteriocin produced by *Lactococcus lactis* subs. *cremoris* to papain has been reported[46]. The bacteriocin produced by the LAB strains did not display any inactivation when treated to papain and were active in the pH range of 4.5-6.0. The ethnic people of the North-East use the indigenous knowledge of fermenting bamboo shoot. The study on the fermentation dynamics during *khorisa* production revealed that the microflora ranged from enterobacteria to yeast.

Conclusion

Different varieties of edible bamboo shoot are consumed all over the world. In India, use of fermented bamboo shoot as a food component is limited to North Eastern region only. *Khorisa* is a rich source of lactic acid bacteria, which can further be exploited for improving the quality of food products. Despite being a rich source of medicinal and nutritional agents, consumption is limited. Improvement of the conventional methods by employing modern scientific technology to upgrade the quality production of fermented bamboo shoot at commercial scale and preserving its unique flavor, aroma, and nutrition is required. Advanced and detailed scientific study of the microflora of fermented bamboo shoots and its bacteriocin producing ability will help in production of fermented products on a large scale leading to preservation for a longer period. The remarkable property of bacteriocin can serve as a potential agent in preserving natural food products without altering the nutritional components.

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