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RESEARCH PAPER

Exiguobacterium aurantiacum mediated fermentation of bamboo shoot, and process optimization for *Soibum* production: A traditional food of Manipur, North-East India

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ABSTRACT

Bamboo shoot are an important traditional food delicacies which are consumed as fresh, fermented, or canned in many Asian countries and is intricately related with human diet. In this study, several bamboo shoot samples (fermented and succulent bamboo shoots) were collected to determine the cynogenic glycoside content, followed by process optimization by using *Exiguobacteruim aurantiacum* FB6-1b, which was used for fermentation at different conditions. *E. aurantiacum* was isolated from bamboo shoot. The cyanide content was analyzed and fermentation process was also evaluated by estimating nutritional parameters of final product. Fresh succulent bamboo shoot had high cyanide content (approx 43 mg/g) compared with fermented samples (22.8-30.4 mg/g). It was observed that boiling the succulent bamboo shoots and fermented shoots removed all the cyanide content. The amount of protein, sugar, amino acid for fermentation of sterilized bamboo shoot with FB6-1b, against fermentation in presence of indigenous bacteria in raw bamboo shoot were found to be (2.2±0.6, 1.3±0.04, 0.38±0.004) mg/g and (2.44±0.03, 1.85±0.3, 0.31±0.006) mg/g, respectively. The nutritional parameter including concentrations of protein, sugar, amino acid were better when fermentation was done at temp 30°C (3.56±0.04, 11.1±0.2, 0.75±0.05 g/100g, respectively) and inoculum size 10^4 cfu/ml (2.57±0.1, 10.5±0.1, 0.27±0.04 g/100g, respectively).

Keywords: cyanide, fermented bamboo shoots, nutritional values

Ethnic people of North-East states, India consume fermented bamboo shoot products as a traditional food (Chauhan *et al.* 2016; Rawat *et al.* 2018). People of Manipur consumed bamboo shoot as fresh or in fermented form, locally known as *Soibum* (Senabati *et al*. 2016; Chauhan *et al.* 2016). It is a highly prized item and its consumption dates back time immemorial. Two types of fermentation methods are in practice classically; *Andro type* and *Noney/Kwatha type*. *Andro type* of preparation of *Soibum* is practiced (only in Andro village) in the bulky roasted earthen pot by fed-batch fermentation and *Noney/Kwatha type*, by batch fermentation which is more acidic in taste and is carried out in traditionally designed bamboo chamber. *Soibum* is produced from shoots of selected species of bamboo as –*Dendrocalamus hamiltonii* (Wanap, Unap, Pecha), *D. sekimensis* and *D. giganteus* (Maribop), *Melancona bambusoide* (Moubi/Muli), *Bambusa tulda* (Utang), *B. balcooa* (chingsaneibi) by natural fermentation (Jeyaram *et al.* 2009). In addition to preservation, fermented foods increases digestibility, improves nutritional and pharmacological values as well. Modern research has revealed that bamboo shoots have a number of health benefits: improving appetite and digestion, weight loss, curing cardiovascular diseases, antioxidant

activities and anti-inflammatory effects (Hu *et al.* 2000) and anti-cancer property (Shi *et al.* 1992). These fermented food are associated with a unique group of microflora which increases the level of proteins, vitamins, essential amino acids and fatty acids (Tripathi *et al.* 1998).

Bamboo, belonging to the family *Poaceae* is a natural resource in the world. It is a plant which is widely distributed and grows wild in the fields and mountains from temperate zone of Japan to the tropical zone of India (Uprethi *et al.* 2001). Size and weight of fresh shoot depends upon climatic, pH and nutrition of soil, rainfall, drainage condition and harvesting period of bamboo shoot (Singh *et al.* 2011b). Due to the presence of cyanogenic glycoside, called taxiphyllin which is toxic in nature, the freshly harvested bamboo shoot are creamy yellow in colour with pungent smell and bitter taste (Schwarzmaier *et al.* 1977). The content of hydrogen cyanide (HCN) varies depending upon the parts of the shoots and the bamboo species, ranging from less than 100 to nearly a 1000 mg HCN per kg of fresh shoot (Poulton *et al.* 1990). When the shoots are disrupted by cutting or peeling, a glycosidase enzyme in the shoots hydrolyzes the glycoside and produces HCN. Generally, traditional preparations method including boiling can reduce the HCN content in bamboo shoots and make them edible (Poulton *et al.*, 1990; Ferreira *et al.* 1995). Bamboo shoots contain 0.3 to 0.8% HCN. Out of which, upto 0.16% of the total cyanide is contained in the tip, reducing to 0.01% in the base, with highest in leaves of young plants, but dropping rapidly after pollination. However, subsequent processing helps in fighting the cyanide concentration, though incomplete cooking result in glycoside hydrolysis and higher release of HCN, but the total amount of HCN in the shoots can be eliminated by cooking for two hours (Tripathi *et al.* 1998; Haque and Bradbury, 2002). For centuries, bamboo shoot have lent unique flavors and a distinctive crunchy texture to the traditional Asian dishes and is easily fermentable, people living in such places have been consuming fermented bamboo shoot as indigenous food (Sharma *et al.* 1989). The viability of integrating raw/processed bamboo shoot in modern diet and lifestyle for enhancing food nutritional security is explored (Nirmala *et al.* 2007).

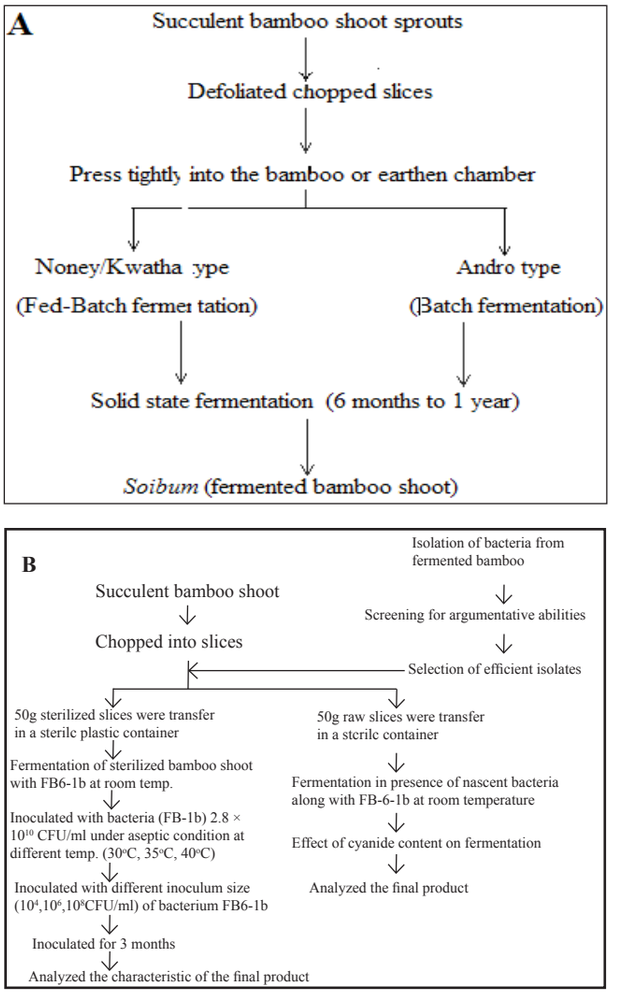

Fig. 1. (A) Flowchart of traditional method for *Soibum* preparation (Jeyaram *et al.* 2009) **(B)** Flowchart of *Soibum* preparation by *in-vitro* fermentation process developed in this study

The preparation techniques of fermented bamboo shoot product varies from region to region, which is known by different traditional names like *soibum* or *soidon* in Manipur, *lung- seij* in Meghalaya, *mesu* in Darjeeling and Sikkim, *bas-tenga* in Nagaland, *ekung/hirring* in Arunachal Pradesh, and *miya-mikhri* by Dimasa tribe in Assam (Das *et al.* 2012). *Soibum*, a unique fermented bamboo shoot food product

of Manipur, showed good quality with a typical characteristics odour and desirable flavor. The product with its exquisite taste and smell serve as a source of protein in the diets of the people (Ross *et al.* 2002). Several researchers have reported describing the nutritional properties of fermented bamboo shoot (Nirmala *et al.* 2008) produce by traditional fermentative process. However, for process development and commercialization of fermented bamboo shoot, there is a need to standardize the process optimization of fermentation by means of bacterial isolate without compromising the nutritional and characteristics of final product. Therefore, the present study was undertaken to optimize *invitro* fermentation of *Soibum* by a suitable bacterial isolate and to determine the cyanogenic glycoside content of both the succulent and fermented bamboo shoot and to investigate their nutritional parameters of the fermented samples.

MATERIALS AND METHODS

Collections of samples

Uniformly size and mature succulent bamboo shoot of *Bambusa balcooa* were procured from Manipur and also fermented samples were collected from its production centre at four valley district places (Bishnupur, Kakching, Lamangdong, Singjamei, Kwatha, Andro) and one hill district (Lamkhang, Kongkhang, Shilet) of Manipur. Manipur lies in the Eastern Himalayan region of India, has a salubrious climate condition with approximate average annual rainfall varying from 933mm at Imphal to 2593mm at Tamenglong and the temperature ranging from sub-zero to 36°C. The study area selected for the present research covers an area of about 2238 sq km. The collected samples were packed in a sterile polythene bags and transported and stored in a laboratory refrigerator for further analysis.

Isolation of microorganism

The samples were then subjected to serial dilution for the isolation of bacteria. The samples were taken in test tube containing 9 ml of sterile water. Suitable dilutions of *Soibum* samples was plated on nutrient agar medium and incubated at 25°C for 48h. Bacterial population was enumerated and pure cultures were isolated for further study by streaking.

Morphological, biochemical and molecular identification of bacteria

5 bacterial isolates were characterized for colony morphology, Gram staining and biochemical test such as catalase, oxidase, Indole production, Methyl red, Voges-proskauer, Citrate utilization, Urease and Triple sugar iron were carried out for initial characterization of bacterial isolated from the fermented samples. Bacterial growth at different pH and different temperatures were also performed. The identification of FB6-1b was done on the basis of 16srDNA gene sequencing MEGA6 software neighbor-joining method with 1000 bootstrap replicates (Tamura *et al.* 2011) PCR techniques was employed using universal primer, 16srDNA: 27F(5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R(5'-TACGGYTACCTTGTTACTT-3'). The taxonomic position and the similarity percentage of the isolates were determined.

Effect of in-vitro fermentation of raw bamboo shoot by 5 different isolates on cynogenic glycoside content

Fermentation was carried out in laboratory with 5 different isolates by a modified form of the traditional method of fermentation (Fig. 1B), the traditional method of preparation is shown in Figure 1A. 50 gram of succulent bamboo of *Bambusa balcooa* shoot were cut into slices and transfer in a sterile polythene which involves inoculating thin slices of raw bamboo shoot. The material was inoculated with selected bacterium under aseptic condition and the samples were kept at room temperature for a period of 90 days, for fermentation. The whole process of fermentation from slicing the shoot till the sealing the polythene bag was taken under sterile condition and the determination of total cyanide in bamboo shoot was done with a modification of Picrate paper kit procedure. In replicate cyanogenic glycocides estimation was done using the technique of the

picrate impregnated paper (Bradbury *et al.* 1999). The new leaf matter of the bamboo shoot was removed exposing the edible bamboo shoot. The tip part contains more cyanide than the middle section which contains more than the base section. Each section was analyzed by cutting a thin section (about 0.5-1 cm thick) across the bamboo shoot and then, cutting a small sector from it. This sector was ground up in a pestle and mortar. (The enzyme breaks down the taxiphyllin quite rapidly to produce HCN, hence these steps were carried as quickly as possible). Immediately weighed out about 25 mg (accurately) each of fermented bamboo shoot samples and boiled. Similarly unfermented and succulent bamboo shoots of the ground shoot were also boiled in a flat bottomed plastic bottle. (Weight of sample = z mg). Immediately added 0.5 mL of 0.1 M phosphate buffer at pH 6. Then, immediately added a yellow picrate paper attached to a plastic strip with precaution that the picrate paper must not touch the liquid in the bottle. Picrate papers were stored at -20°C. The bottle was closed with a screw capped lid. Another sample as above but with no bamboo shoot was processed to serve as a blank. The bottles were allowed to stand for 16-24 hour at room temperature (20-35^{o} C). The picrate paper was immersed in 5.0 ml of water (measured accurately with a pipette) for about 30 min with occasional gentle shaking. Took the blank picrate paper, removed its plastic sheet and immersed the yellow picrate paper in 5.0 ml of water for about 30 min with occasional gentle shaking. The absorbance were measured at 510 nm of the picrate solution against the blank. The total cyanogen content in ppm was calculated by the equation given below:

Total cyanogen content (ppm) = 396 × absorbance x 100 / z.

Fermentation of sterilized bamboo shoot with FB6-1b, and fermentation in presence of indigenous bacteria in raw bamboo shoot

Fermentation was carried out in laboratory by a modified form of the traditional method of fermentation (Fig. 1B). The fresh succulent bamboo shoot of *Bambusa balcooa* were chopped it into slices and weigh 50 gram of slices succulent bamboo shoot and transferred it in a sterile polythene bag which involves inoculating thin slices of raw bamboo shoot. 2 ml of 1 O.D of bacteria was inoculated with each, raw bamboo shoot as well as sterilized bamboo shoot, under aseptic condition and the samples were kept at room temperature for a period of 90 days, for fermentation. The whole process of fermentation from slicing the shoot till the sealing the polythene bag was taken under sterile condition in triplicate.

Fermentation by using FB6-1b at different temperatures

Fermentation was carried out under laboratory condition at different temperature (i.e. 30 ^{o}C, 35 ^{o}C, 40 ^{o}C) with a slight modification of traditional method. 50 gram of succulent bamboo shoot inoculum of 2.8×10^{10} CFU/ml bacterial culture in late log phase was used as inoculum for fermentation and was carried out triplicate in laboratory by inoculating FB6-1b with the raw bamboo slices in a sterile plastic container and incubated at 30 ^{o}C, 35 ^{o}C, 40 ^{o}C respectively for 90 days.

Effect of inoculum size on fermentation

The inoculum of bacteria was prepared in Nutrient broth (NB). The loopful of culture were inoculated and incubated on rotatory shaker for 24 hour at 25° C. The pallet were collected at 1000 rpm for 10 mins and washed thrice with sterile distilled water. The cells were suspended in sterile water to obtain population of 10^4, 10^6 and 10^8CFU/ml as confirmed later by enumerating the CFU on NA plates. The fermentation process was carried out as described earlier.

Physico-chemical and characteristic of fermented bamboo shoot

Sample (1.0 gram) of fermented bamboo shoot was blended in 10ml of sterile water and the pH was determined using a pH meter (Type 335, Systrinocs), as per the standard procedure (AOAC, 1999).

Chemical composition such as protein by (Lowry *et al.* 1951), carbohydrate as sugar (Sadashivam and Manikam, 1985), amino acid (Sadashivam and

Manikam, 1985), moisture content, ash content, colour and nature was determined adopting the standard procedure. Moisture content was calculated by drying the sample to a constant weight. Ash content was measured by heating the sample at 600°C until the difference between two successive weighing was < 1 mg (AOAC, 1999).

Experimental and Statistical analysis

The experiment has 3 sets of treatment (Sterilized and non-sterilized *in-vitro* fermentation, *in-vitro* fermentation at different temperature, *in-vitro* fermentation at different inoculum size) each inoculated with the bacteria along with the control (without inoculum). The biochemical and microbial count were also analyzed. The values were expressed as mean ± standard deviation (SD) and the data were processed using Microsoft Exel.

RESULTS AND DISCUSSION

Isolation of bacteria and screening for their fermentative ability and cyanogen content

A total of 88 bacterial isolates were isolated from *Soibum* samples. These isolates were checked to ferment raw bamboo shoots and cyanide content in final product. Results of five efficient bacterial isolates i.e., FB2-4, FB2-10a, FB6-1b, FB6-3, FB6-6 that reduced cyanogen content of raw bamboo shoot are given in Fig. 2.

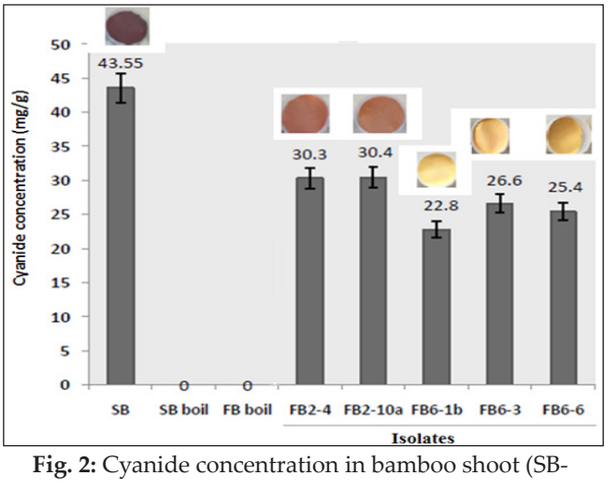

Fig. 2: Cyanide concentration in bamboo shoot (SB- Succulent bamboo shoot, SB boil, FB boil- Fermented bamboo shoot boil)

The unfermented raw - succulent bamboo shoot had high cyanide content (approx. 43.55 mg/g), as compared to fermented bamboo shoots (22.8 -30.4 mg/g). The cyanide content of final product of fermented *Soibum* was found to be the lowest with FB6-1b (22.8 mg/g) followed by FB6-6 and FB6-3 (25.4 mg/g and 26.6 mg/g respectively). Therefore, Fb6-1b was selected for process optimization of fermentation process. Boiling the succulent bamboo shoot and fermented bamboo shoots completely eliminated/ removed the cyanide from all the samples (Fig. 2).

Microbiological characterization of Exiquobacterium aurantiacum, FB6-1b

Several bacterial were isolated from *Soibum* samples and checked for *in-vitro* fermentative process. The succulent bamboo contains cyanogenic glycosides which makes it harmful for human consumption. Cyanogenic glycoside content was dectected in all the samples, though the content was very less in fermented bamboo shoot, as compared to unfermented raw succulent bamboo shoot. Also boiling completely removed the cyanogenic glycosides from all the samples. It is known that there is loss of cyanogens content during the traditional process of fermentation which includes, chopping of tender shoots into pieces, partial drying of fresh shoots, boiling in water or salt water and draining or keeping the tender shoots in hot water for 10-15 min (Schwarzmaier, 1977). Cyanogenesis is the natural phenomenon occurs in plant to prevent themselves against their enemies. Many edible plants contain cyanogenic glycosides, whose concentration varies widely as a result of location, season, genetic, soil type and environment factors. A WHO (1993) reports states that the concentration of cyanide in immature shoot tip of bamboo is 8000mg/kg of hydrogen cyanide. (Haque and Bradbury, 2002) reported that the cyanide content ranges from 100-1600ppm, but (Ferreira *et al.* 1990) reported it to be as much as 1000mg/kg of hydrogen cyanide in the epical part. Fermentation is the best way to reduced cyanogenic

glycosides of fermented bamboo shoot samples making it more favorable for human consumption. The microbial community that inhabits *Soibum* has not been optimized for degradation of cyanogenic glycosides. (Vetter *et al.* 2000) reported that the biosynthetic precursors of the cyanogenic glycosides are different L-amino acids, which are hydroxylated, then the N-hydroxylamino acids are converted to aldoximes and these are converted into nitriles and hydroxylated to alpha-hydroxy nitriles and then glycosylated to cyanogenic glycosides. Therefore fermentation by another strategy to improve the food nutritive value, which eliminates the cyanogenic glycosides content in bamboo shoots besides increasing its flavor and texture, is required. Among all the fermented samples, *E. aurantiacum* FB6-1b showed better fermentative abilities than the other isolates. Therefore, FB6-1b was selected for further analysis.

FB6-1b was Gram positive rods, forming light orange, circular, smooth colonies on Nutrient Agar. Optimum growth was obtained at pH 7 at 30 °C. The phenotypic and physiological characteristic of isolate is given at Table 1.

Table 1: Morphological and physiological characteristics of *Exiguobacterium aurantiacum* from fermented bamboo shoots

Morphological	**FB6-1b**
Colony morphology	Circular margin, Light orange, Entire, Raise
Gram	+ve
Cell morphology	Cocci in cluster form
Biochemical characteristics	
Indole	+
MR	+
VP	–
Catalase	+
Oxidase	+
TSI	A/A
Citrate	–
Urease	+
Physiologcal characteristics	
Growth OD at different pH	
4	0.308
5	0.340
6	0.380
7	0.517
8	0.376
9	0.321
Growth OD at temperature	
30° C	0.376
35° C	0.138
40° C	0.118
Growth OD at different salt concentration	
0.5%	1.304
2%	1.544
5%	0.914
10%	0.236

'+'*Positive & '' Negative,* **'+++'** *Excellent Growth, '++' Moderate Growth, '+' Less Growth, '' No Growth.*

The BLAST search of 16s rRNA gene sequences indicated FB6-1b confirmed it to be *Exiguobacterium aurantiacum.* On the basis of phylogenetic tree constructed with the 16RNA similarity (%), it was identified as *E. aurantiacum* and maximum similarity was observed with isolate DSM 6208T/JNIQ01000001 (Fig. 3). The phylogenetic tree was clustered in two different groups. The isolate FB6-1b was separately clustered with other nearest related *Exiquobacterium* taxa. FB6-1b was found to be closely associated with *Exiquobacterium aurantiacum* DSM 6208T/ NIQ01000001.

Optimization of fermentation parameter and process

Fermentation of sterilized bamboo shoot with FB6-1b, in presence of indigenous bacteria in raw bamboo shoot

The average protein content of sterilized fermented samples inoculated with FB6-1b as starter culture was found to be 2.2±0.6 (g/100g) on wet weight basis and that of the control (unfermented) was 1.8±0.2 (g/100g) while the protein content when fermented in presence of indigenous bacteria raw bamboo shoot was 2.44±0.03 and the control was 1.75±0.03 (fermented with indigenous bacteria) (Table 2). For

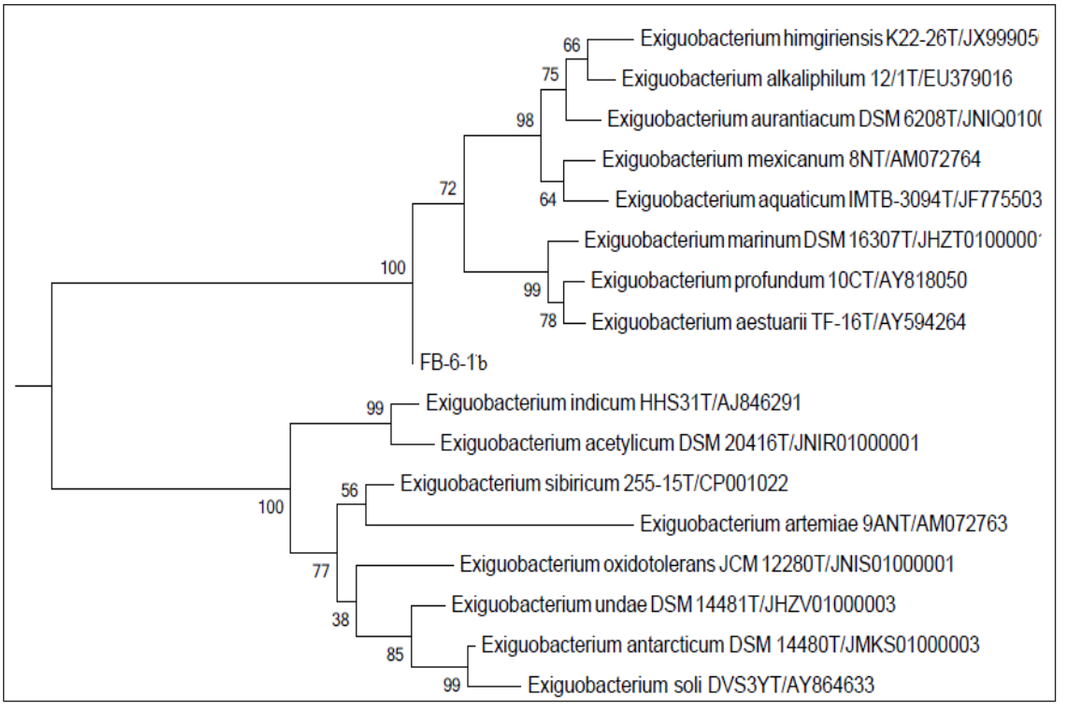

Fig. 3: Phylogenetic analysis of 16sDNA gene sequence of the bacterial FB6-1b isolate isolated from fermented bamboo shoot. The analysis was conducted with MEGA6 using neighbor joining method

Fig. 4.(A) Bamboo shoot before fermentation. **(B)** Fermented bamboo shoot.

FB6-1b fermentation of otherwise sterilized bamboo shoots, the sugar content was found to be 1.31±0.04 (g/100g), while in control (unfermented) it was 2.6±0.1 (g/100g). However the sugar content on fermentation with indigenous bacteria in raw bamboo shoot was 1.85±0.03 and the control (fermented with indigenous bacteria) was 3.0±0.06. The amino acid content was recorded as 0.38±0.004 (g/100g) and 0.52±0.5 (g/100g) in FB6-1b sterilized fermented *Soibum* and control (unfermented) respectively while the amino acid

Table 2: Nutrients composition of fermentation of sterilized bamboo shoot with *Exiguobacterium aurantiacum* FB6-1b, against fermentation in presence of indigenous bacteria in raw bamboo shoot (g/100gm)

In-vitro fermentation	Nutrient Composition						pH	Moisture	Ash
	Sample	Protein	Sugar	Amino acid	Nature	Colour			
Sterilized	Control	1.8±0.2	2.6±0.1	0.52±0.5	Non sticky	Cream	4.1	88.3	2.2
	FB6-1b	2.2±0.6	1.31±0.04	0.38±0.004	Sticky	Dark cream	3.7	89.1	2.0
Non-Sterilized	Control	1.75±0.03	3.0±0.06	0.46±0.001	Non sticky	Cream	4	88.1	2.1
	FB6-1b	2.4±0.03	1.85±0.3	0.31±0.006	Sticky	Yellowish	3.6	89	1.8

Note: *All value are expressd in g/100g wet basis, except for pH, moisture and ash content; Data represents the mean scores ± SD(n=3).*

content on fermentation with indigenous bacteria in raw bamboo shoot was 0.31±0.006 and the control (fermented with indigenous bacteria) was 0.46±0.001.

Effect of temperature on fermentation

In the present study, the nutrient composition of final product obtained after *in vitro* fermentation at 30^{0}C using *E. aurantiacum* FB6-1b (Table 3), show that the average protein content of fermented samples was 3.56±0.09 (g/100g) on wet weight basis and that of the control was 2.47±0.2(g/100g) when inoculated with FB6-1b as starter culture. The sugar content was found to be 11.1±0.01 (g/100g) for FB6-1b fermented product, while the control was 11.04±0.2 (g/100g). The amino acid content was recorded as 0.75±0.005 (g/100g) and 0.69±0.06 (g/100g) in FB6-1b fermented *Soibum* and control respectively. The average moisture content was 86.6% and 88.1%, while ash content was 1.8% and 2.0% for FB6-1b fermented *Soibum* and control respectively.

For *in vitro* fermentation at 35 ^{o}C, when inoculated with FB6-1b as starter culture the average protein content of fermented samples were found to be 2.1±0.2 (g/100g) on wet weight basis and that of the control was recorded as 1.62±0.01 (g/100g). The sugar content was 11.48±0.01 (g/100g) for FB6-1b fermented product while in control it was 11.63±0.1 (g/100g). The amino acid content was found to be 0.31±0.02(g/100g) and 0.43±0.02 (g/100g)for FB6-1b fermented product and control respectively. The average moisture content was 86.1% and 90.2% while ash content was 1.9% and 2.0% for FB6-1b fermented *Soibum* and control respectively.

For *in vitro* fermentation at 40 ^{o}C, after inoculated with FB6-1b as starter culture the average protein content of fermented samples was found to be 3.48±0.1 (g/100g) on wet weight basis and that of the control was 3.56±0.4 (g/100g). The sugar content was 9.6±0.1 (g/100g) for FB6-1b fermented product while the control was 9.38±0.03 (g/100g). The amino acid content was 0.097±0.1 (g/100g) and 0.27±0.01 (g/100g) for FB6-1b fermented product and control respectively. The average moisture content was 86% and 89.1% while ash content was 1.9% and 2.0% for FB6-1b fermented *Soibum* control respectively.

Table 3: Comparative account of nutrients (g/100gm) of fermented bamboo shoot obtained by *in vitro* fermentation at different temperatures

In-vitro Fermentation	Nutrient composition						pH	Moisture	Ash
	Sample	Protein	Sugar	Amino acid	Nature	Colour			
Fermentation at 30^{o}C	Control	2.47±0.2	11.04±0.2	0.69±0.06	Non-sticky	Cream	4	88.1	2.0
	FB6-1b	3.56±0.4	11.1±0.2	0.75±0.05	Sticky	Cream	3.6	86.6	1.8
Fermentation at 35^{o}C	Control	1.62±0.01	11.63±0.1	0.43±0.02	Non-sticky	Cream	4.1	90.2	2.0
	FB6-1b	2.1±0.2	11.48±0.1	0.31±0.04	Thick sticky	Yellow	3.8	86.1	1.9
Fermentation at 40^{o}C	Control	3.56±0.04	9.38±0.03	0.27±0.01	Non-sticky	Cream	4	89.1	2.0
	FB6-1b	3.48±0.2	9.6±0.3	0.097±0.01	Non-sticky	Whitish	3.7	86.0	1.9

All values are expressed in g/100g wet basis, except for pH, moisture and ash content; Data represents the mean scores ± SD (n=3).

Thus, the best nutritive values were obtained when fermentation was carried out at 30° C using *E. aurantiacum* FB6-1b.

Effect of different inoculum sizes of Exiguobacterium aurantiacum FB6-1b on fermentation

In vitro fermentation with inoculum size of 10^4 CFU/ml of FB6-1b as starter culture the average protein content was found to be 2.57±0.1 (g/100g) in weight wet basis in fermented samples while the control was 2.38±0.1 (g/100g). The sugar content was found to be 10.5±0.1 (g/100g) while the control was 10.9±0.3 (g/100g). The amino acid content was 0.27±0.01 (g/100g) and 0.45±0.001 (g/100g) for FB2-4fermented product and control respectively. The average moisture content was 86.1% and 88.3% while ash content was 1.8% and 1.9% for FB6-1b fermented product and control respectively.

In vitro fermentation for inoculum size 10^6 CFU/ml, the average protein content of fermented samples using FB6-1b inoculum as starter culture was 2.44±0.02 (g/100g) on wet weight basis and the control was 2.38±0.1 (g/100g). The sugar content was 10.6±0.06 (g/100g) for FB6-1b fermented product whereas for the control it was 10.92±0.3 (g/100g). The amino acid content was found to be 0.17±0.03 (g/100g) and the control was 0.45±0.001 (g/100g). The average moisture content was 86.1% and 88.1% while ash content was 2.0 and 2.1% for FB6-1b fermented product and control respectively.

In vitro fermentation for inoculum size 10^8 CFU/ml, the average protein content of fermented samples using FB6-1b inoculum as starter culture was found to be 2.0±0.009(g/100g) on wet weight basis and the control was 2.38±0.1 (g/100g). The sugar content was 12.3±0.2 (g/100g) whereas control was 10.92±0.3 (g/100g). The amino acid content was found to be 0.16±0.02 (g/100g) for FB6-1b fermented product and 0.45±0.001 (g/100g) for the control, respectively. The average moisture content was 87.1% and 89.1% while ash content was 1.9% and 2.0% for FB6-1b fermented product and control respectively.

Therefore, the best nutrient composition was observed on *in-vitro* fermentation at 10^4 CFU/ml inoculum size in table 4. The nature and colour of final product of all the fermented samples were sticky, dark cream, non-sticky, yellowish, cream, brownish while non-sticky, cream for control sample.

In fact, fermentation of bamboo shoots in controlled environments has not been attempted earlier and thus optimization was carried out using *E. aurantiacum* FB6-1b. However, several researchers have been reported on analysis of nutritive value of bamboo shoot, fermented by traditional methods. Bamboo shoot contains about 88.8 % water, more than 3.9% protein and 17 amino acids (Satya *et al.* 2009b; Nirmala *et al.* 2007). Collins *et al.* (1983) was first to describe *E. aurantiacum* strain DSM6208T from a potato processing plant. Since then, it has been reported from diverse habitats such as ancient permafrost in Siberia, and Yellowstone park (Vishnvetskaya *et al.* 2009). They have the ability to grow in extreme temperatures, some strains are halotolerent. This is

Table 4: Comparative account of nutrients (g/100gm) of fermented bamboo shoot obtained by *in vitro* fermentation at different inoculum size

In vitro Fermentation		Nutrient Composition					pH	Moisture	Ash
	Sample	Protein	Sugar	Amino acid	Nature	Colour			
Inoculum size, 10^4	Control	2.38±0.1	10.9±0.3	0.45±0.001	Non-sticky	Cream	4.1	88.3	1.9
	FB6-1b	2.57±0.1	10.5±0.1	0.27±0.04	Sticky	Cream	3.7	86.1	1.8
Inoculum size, 10^6	Control	2.38±0.01	10.92±0.3	0.45±0.001	Non-sticky	Yellow	4	88.1	2.1
	FB6-1b	2.44±0.01	10.6±0.06	0.17±0.03	Non-sticky	Cream	3.8	86	2
Inoculum size, 10^8	Control	2.38±0.1	10.9±0.3	0.45±0.01	Non-sticky	Cream	4	89.1	2.0
	FB6-1b	2.0±0.009	12.3±0.2	0.16±0.03	Sticky	Whitish	3.9	87.1	1.9

All value are expressed in g/100g wet basis, except for pH, moisture and ash content. Data represents the mean scores ± SD (n=3).

the first report that *E. aurantiacum* is isolated from fermented bamboo shoot samples, and till now, there is no report where *E. aurantiacum* has been used for food fermentations.

In this study, the nutrient composition of final product obtained after *in vitro* fermentation of sterilized fermented bamboo shoot with FB6-1b was much better than the product obtained by fermentation in presence of indigenous bacteria. The pH of non-fermented bamboo shoot was around 6.0. but when autoclaved it decreased up to 4.0. Further, after *in-vitro* fermentation the pH was reduces between 3.4-3.7. It was reported earlier that the pH of final product remains around 3.6, when prepared by traditional process (Hoque *et al.* 2010). The average moisture content was 89.1% and 88%, while ash content was 2.2% and 2.0% for FB6-1b fermented *Soibum* and the control respectively whereas the moisture content on fermentation with indigenous bacteria in raw bamboo shoot and the control was 89 % and 88.1 % and ash content was 1.8 and 2.1, respectively. Choudhury *et al.* (2011) reported that the moisture content in fermented bamboo shoot remains in range of 31% -52%, as compared with raw bamboo of 80%. Total ash content in fermented sample was higher (6.22-14.36%) in comparison to raw shoots (1.11%).

Although, the protein content of sterilized fermented sample inoculated with FB6-1b as starter along was increased by 22% and that of fermented samples with indigenous bacteria was 39% as compared with the control. The sugar and amino acid content was decreased 49% and 26% for FB6-1b fermented product, while however the sugar and amino acid content on fermentation with indigenous bacteria was 38% and 32% respectively. Thus, the final product was found better for nutritional parameter when sterilized bamboo shoots were fermented with *E. aurantiacum* FB6-1b.

The final product was found comparatively enhanced for nutritional contents when fermented at 30 °C, comparing with 35 °C and 40 °C. As a matter of fact, the protein and amino acid content was increased by 44% and 8%, while sugar content was decreased by 2% respectively, comparing with the control. 29% of protein content was found increase in fermentation at 35° C comparing with control whereas fermentation at 40° C resulted decreased up to 2% of the protein content which evidently shows that fermentation process was extremely susceptible to variant in temperature.

Though, at inoculum size of 10^4 CFU/ml, protein content of final product was higher by 7% than non-fermented control, also the amino acid content was decreased by 40% while at inoculum size 10^6 CFU/ml, protein content was 2% increased while sugar and amino acid reduced upto 2% and 62% respectively as compared to non fermented control. Further, on increasing the inoculum size the nutritive value of final product was found to be reduced. Therefore, the result indicates that lower inoculum size results in superior fermentative process with *E. aurantiacum* FB6-1b. In traditional method, the fermentation is usually performed at room temperatures, where the process has to resist the undesirable fluctuations, and therefore, there is variation in nutritional quality of final product. In fact, temperature has been considered an important factor on final product of fermentative foods like fermented soybean (*Hawaijar*), where 25° C - 40 °C has been reported to be optimum (Tamang *et al.* 2009). Though, there is no such report available for fermented bamboo shoots.

However, Ferreira *et al.* 1990, and Choudhury *et al.* 2011 reported that protein, amino acid and carbohydrate contents in fermented bamboo shoot prepared by traditional process were 2.17(g/100g), 2.005 (g/100g) and 1.504 (g/100g), respectively, as compared to non-fermented fresh shoot 3.108 (g/100g), 3.863 (g/100g) and 5.103 (g/100g). The controlled fermentation using *E. aurantiacum* FB6-1b resulted in protein, sugar and amino acid contents of 3.56 (g/100g), 11.1 (g/100g), and 0.75 (g/100g) respectively, which reflects improved fermentative process than traditional fermentation. Increase in soluble protein content with increase in fermentation period has been suggested for fermented bamboo shoots (Satya *et al.* 2010), even though for significant comparison of data, the controlled fermentation was carried out for duration similar to traditional process.

(Kumbhare and Bhargava, 2007) reported that the content of protein decreased upon boiling the bamboo shoot by 25% due to denaturation of protein. In the traditional fermentation process, the initial materials of fresh bamboo shoots are not boiled, and therefore, consumption of raw fermented bamboo shoots is not recommended (Nirmala *et al.* 2008). Further, boiling the initial material does not affected the protein content of final product. This degradative process however brings out certain characteristics flavour that is essential for the quality of the final product. The increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles. Some other researchers have also examined the amino acid content and it was found the total free amino acids were found to decrease in case of processed shoot (Kumbhare and Bhargava, 2007). The amino acids degrades after prolong heating at high temperature. This may be the possible cause for decrease in amino acid content. The major change in amino acids that occurs on cooking is due to Maillard reaction which makes lysine unavailable, thereby reducing nutritive value (Meredith *et al.* 1979). Carbohydrates had been reported to be in range of 2.6-3.9 (g/100g) fresh wt, in raw non-fermented shoots of some bamboo species. The content increases after boiling (5.1, 5.0, 5.0 and (3.1 g/100g) respectively, which might be due to fibrous nature of bamboo (Kumbhare and Bhargava, 2007) but a substantial decrease had been reported after fermentation by traditional process, which was in accordance to present findings with controlled fermentation (Tamang *et al.* 1996). This is because polysaccharides could have been hydrolyze into simple sugars and resultant monosaccharides contribute to this increase (Kumbhare and Bhargava, 2007). Fermentation has been shown to reduce the amount of reducing sugar to great extent converting them to acid resulting in the rise of acidity till reducing sugar bio-conversion get exhausted (Singh *et al.* 2011).

CONCLUSION

In North-Eastern India, indigenous food fermentations are carried out spontaneously without adding any starter cultures in uncontrolled environments. Therefore *in-vitro* fermentation of bamboo shoot was carried out in controlled environment with starter culture *E. aurantiacum* FB6-1b. This study confirmed improvement in product quality with better nutritional characteristics of fermented bamboo shoot. Fermentation resulted in sufficient decrease of cyanogenic glycosides of *Soibum* samples making it suitable for human consumption. pH, temperature and fermentation period were optimized with objective of process development for commercialization. Fermentation in controlled environment using *E. aurantiacum* FB6-1b enhanced food quality by reducing the process duration, making it more nutritious and health risk free, maintaining better quality and flavor.

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