



MICROBIOLOGICAL STUDIES ON HUNGRII, RHUJUK/BASTANGA AND TSUTUOCIE- FERMENTED FOOD PRODUCTS OF NAGALAND, INDIA

BENDANGNARO JAMIR AND CHITTA RANJAN DEB*

Department of Botany, Nagaland University, Lumami-798627, Nagaland, India

ABSTRACT

Nagaland is inhabited by diverse tribal communities and it produces varieties of fermented foods, which so far least explored. Fermented food products in Nagaland are still produced from spontaneous and uncontrolled fermentation and thus it results in a product of variable quality. In, this study, the vegetable based fermented food products i.e., *Hungrii* (*Brassica* leaves), *Rhujuk/Bastanga* (Bamboo shoot) and *Tsutuocie* (Cucumber) have been studied for their microbial population. Microbiological analysis of the fermented products revealed the presence of *Bacillus* species in almost all the fermented food products. Food contaminant like *Staphylococcus* was detected in the fermented product, as fermentation in Nagaland is still carried out at the household levels using traditional methods.

KEYWORDS: *Nagaland, Fermented foods, Hungrii, Rhujuk/Bastang, Tsutuocie.*



CHITTA RANJAN DEB*

Department of Botany, Nagaland University,
Lumami-798627, Nagaland, India

Received on: 04-07-2018

Revised and Accepted on: 16-08-2018

DOI: <http://dx.doi.org/10.22376/ijpbs.2018.9.4.b11-17>



[Creative commons version 4.0](https://creativecommons.org/licenses/by-nc-sa/4.0/)

INTRODUCTION

Foods are considered to be perfect media for the growth of microorganisms, sometimes rendering it to be inedible and dangerous for consumption. Intriguingly, not all microorganisms are foes, since they can convert raw food materials into palatable, analgesic and mentally stimulating food products with flavors, aroma and texture pleasant for human consumption^{1,2}. A wide spectrum of microorganisms is involved during fermentation processes but only a few types usually determine the quality of the end product³. Microorganisms may be present as natural indigenous microbiota in uncooked plant or animal substrates, utensils, containers, earthen pots, or environment or as added starter cultures containing functional microorganisms⁴. Fermentation of fruits and vegetable products began started from the time ancient people started collecting and storing food. Perishable and seasonal leafy vegetables, cucumbers including young edible tender bamboo shoots are traditionally fermented into edible products using the indigenous knowledge of bio preservation^{5,6}. *Hungrii*, *Rhujuk/Bastanga* and *Tsutuocie* are vegetable based fermented food products of Nagaland. They are commonly consumed as a condiment. Fermented condiments are used as taste enhancers in traditional dishes⁷. *Hungrii* is a fermented product prepared from mustard leaves. Pit-fermentation method is followed during its preparation. The leaves are sun dried, pressed tightly into the pit and covered or plastered with mud on top. Natural fermentation takes for about 15-18 days, after which it is sun dried again to get the final product. It can be stored for 2-3 years (Figure 1A). *Rhujuk/Bastanga* is prepared from succulent bamboo shoots. The sheaths are removed, cut into small pieces and slightly pounded. They are then put into bamboo basket, with hole in the middle and kept pressed tightly for about 2-3 weeks till they completely get drained of its juice. The fermented product can be stored for years (Figure 1B). *Tsutuocie* is a cucumber based fermented product. For its preparation, seeds are removed from the matured cucumbers and cut into small pieces. They are put into jars or earthen pots along with some amount of water and allowed to ferment naturally for about 3-4 months. It can be kept for over 5-6 years (Figure 1C). In Nagaland, fermented foods are still prepared traditionally at household level. Natural or spontaneous fermentation usually occurs during the preparation of the various traditional fermented products, which leads to occurrence of mixed microbial populations and difference in product quality^{8,9}. Present study was undertaken to isolate the dominant microorganisms present in the final fermented food product and to identify them using DNA- based identification method.

MATERIALS AND METHODS

Material

Samples were collected randomly from different households from in and around Nagaland. Samples were kept in a refrigerator at 4°C until processing.

Methods

Estimation of pH

Five grams of sample were mixed and homogenized

with 10 ml distilled water and pH was measured using a digital pH-meter.

Microbiological methods

Ten grams of sample were taken aseptically and homogenized in sterile physiological saline [peptone (0.1%, w/v); NaCl (0.85%, w/v)] for 1 min. Then serial dilutions were prepared by transferring one ml from first dilution (10^{-1}) to 9 ml peptone water and serially diluted further up to 10^{-10} dilutions with saline water. Then plate counts were carried out using the following media, temperature and incubation periods to enumerate different microbial group.

Total viable bacteria count

To determine the total bacterial count 0.1 ml of serially diluted 0.1% (w/v) sample was inoculated plate count agar (PCA) and incubated at 30-32°C for 48h. Colony forming units (CFU) were counted using a colony counter and the results were presented as cfu ml⁻¹.

Enumeration of coliform bacteria

Appropriate decimal dilutions (0.1 ml) of the homogenate was spread on Nutrient Agar and Tryptone Soya Agar and was incubated at 37°C for 24 h. Members of Enterobacteriaceae were enumerated using Violet red bile glucose agar and incubated at 30°C for 48 h.

Enumeration of lactic acid bacteria

From appropriate dilutions, 0.1 ml aliquots were spread plated in triplicates on pre-dried surfaces of MRS (de-Mann, Rogosa and Sharp) agar plates supplemented with calcium carbonate(1%, w/v). The plates were incubated anaerobically in an Anaerobic Gas-Bag system at 30-32°C for 48h.

Enumeration of Staphylococci

Selective enumeration was carried out by spread plates on Baird-Parker agar media. The plates were incubated at 37°C for 48 h.

Yeast and mold enumeration

From suitable dilution of sample, 0.1 ml was transferred onto solidified potato dextrose agar and yeast malt agar, supplemented with 12 µg ml⁻¹ Streptomycin to inhibit bacterial growth. Plates were then incubated at 27°C for 48 h.

Phenotypic characterization

Morphologically different colonies were isolated and purified cultures were grown on slants of the same medium and stored at 4°C. Purified isolates were checked for gram stain and for catalase production.

Molecular identification

DNA isolation

Extraction of genomic DNA was done using CTAB protocol¹⁰. About 5 ml bacterial broths was centrifuged at 10,000 rpm for 5 min at 4°C followed by suspended in 500µL of TE buffer and thoroughly mixed with 200µL of Lysozyme. The mixture was incubated for 45 min at 37°C water bath. To this 10µL of proteinase K and 50µL of SDS were added and mixed thoroughly and

incubated at 37°C until the solution becomes clear and viscous. Subsequently, 100µL of 5M NaCl was then added and incubated at 65°C for 5 min. It was again incubated at 65°C for 10 min after addition of 100µL CTAB solution. The suspension was extracted with equal volumes of phenol: chloroform: iso-amyl alcohol (25:24:1) and centrifuged at 10,000 rpm for 10 min. The upper phase was transferred and to it equal volume of chilled isopropanol was added and mixed thoroughly by inverting the tubes. Aqueous phase was recovered by centrifugation at 10,000 rpm for 15 min. Isopropanol was removed and the pellet was washed in 70% ethanol by centrifugation at 10,000 rpm for 15 min. The pellets were then allowed to stand for 5-10 min and then re-suspended in 50µL of TE buffer. The extracted genomic DNA was tested qualitatively on 1% (w/v) agarose gel electrophoresis and quantified using Nanodrop Spectrophotometer.

Polymerase Chain Reaction (PCR)

The PCR was performed in a thermal cycler under the following standardized conditions. The 16S rDNA gene sequences were amplified using universal primers 9F (5'-CGCGGGATCCGAGTT TGATCCTGGCTC-3') and 1492R (5'-GGCCGTCGACACGGA TACCTTGTTACGACTT-3') and 27F (5'-AGAGTTTGTATCCTGGCTCAG-3') and 1492R (5'-GGCTACCTTGTTACGACTT-3')^{11,12}. About 25µL of PCR mixture was amplified in a PCR programmed with the following temperatures: 94°C for 5 min then 35 cycles at 94°C 1 min, 60°C for 1 min and 72°C for 30 sec. The final extension was at 72°C for 5 min and stopped at 4°C.

16S rDNA sequencing

Amplified products were separated by electrophoresis in 1.2% (w/v) agarose gel and were purified using a commercial kit (HiPura PCR Product Purification Kit, Make: HiMedia, India). Sequencing was done at 1st Base, Singapore. To determine the closest known relatives of the partial DNA sequences obtained, searches for homologous nucleic acid sequences was performed using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/>). Percent similarity values of the most closely related identities were determined by a comparison with the sequences available in the database using BLAST software¹³.

RESULTS

pH and microbial count

Microorganism needs particular environment to survive and grow, thus pH value is an important factor upon which microbial population can be determined. The pH levels of *Hungrii* and *Rhujuk/Bastanga* were observed to be acidic (5.2 and 4.7 respectively). Whereas, *Tsutuocie* was found to be alkaline in nature (8.2). The total microbial loads of *Hungrii* and *Tsutuocie* were in the range of 10⁴ cfu ml⁻¹, and for *Rhujuk/Basatnga*, it was in the range of 10⁷ cfu ml⁻¹. Yeast and molds were not detected in any of the samples.

Identification

The isolates from all the three products after gram staining were found to be mostly gram negative, spore formers and catalase positive belonging to the genus *Bacillus*. One of the isolate isolated from *Rhujuk/Bastanga* was found to be gram positive rod belonging to *Staphylococcus* species. Microbial species present in *Hungrii*, *Rhujuk/Bastanga* and *Tsutuocie* were identified after comparing their sequence data to sequences listed in the NCBI database are given in Table 1, 2, 3. Sequencing of the 16S rRNA gene of the isolates from *Hungrii*, confirmed that they belonged to the *Bacillus* group of bacteria. Sequence of the isolate BJ-DEBCR- 19, 26, 36 were 98-100% identical to the 16S rRNA gene of *Bacillus licheniformis*. Whereas, isolates BJ-DEBCR- 11, 23, 35 were 98-99% identical to *Bacillus pumilis*, *Bacillus subtilis* and *Bacillus amyloliquefaciens* respectively. Sequencing of the 16S rRNA gene of the isolates from *Rhujuk/Basatnga*, confirmed they belonged to the *Bacillus* group of bacteria. Sequence of the isolate BJ-DEBCR- 9, 14, 30, 31, 37, 39 were 97-99% identical to the 16S rRNA gene of *Bacillus subtilis*. But, isolates BJ-DEBCR- 5, 38, 32 were 97-99% identical to *Bacillus licheniformis* and *Bacillus amyloliquefaciens* respectively. However, one of the isolate BJ-DEBCR-37 was found to be 99% identical to *Staphylococcus* species. Sequencing of the 16S rRNA gene of the isolates from *Tsutuocie*, confirmed that they belonged to the *Bacillus* group of bacteria. Sequence of the isolate BJ-DEBCR- 7, 8, 25 were 98-99% identical to the 16S rRNA gene of *Bacillus subtilis*. Subsequently, isolates BJ-DEBCR- 10, 12, 34 were 98-99% identical to *Bacillus licheniformis* and *Bacillus pumilis* respectively.

Table 1
16S rRNA sequence based identification of microbes from *Hungrii* with GenBank accession numbers

Sl. No.	Isolates	Closest related microorganism	Max. score	Query (%)	E value	Similarity (%)	GenBank Accession No.
1	BJ-DEBCR-11	<i>Bacillus pumilis</i>	1249	94	0.0	99	KU301334
2	BJ-DEBCR-19	<i>Bacillus licheniformis</i>	1400	100	0.0	100	KX258615
3	BJ-DEBCR-26	<i>Bacillus licheniformis</i>	1290	99	0.0	98	MF487824
4	BJ-DEBCR-36	<i>Bacillus licheniformis</i>	1400	100	0.0	100	MF487834
5	BJ-DEBCR-23	<i>Bacillus subtilis</i>	1773	99	0.0	99	MF487821
6	BJ-DEBCR-35	<i>Bacillus amyloliquefaciens</i>	1469	98	0.0	98	MF487833

Table 2
16S rRNA sequence based identification of microbes from Rhujuk/Bastanga with GenBank accession numbers

Sl. No.	Isolates	Closest related microorganism	Max. score	Query (%)	E value	Similarity (%)	GenBank Accession No.
1	BJ-DEBCR-9	Bacillus subtilis	953	91	0.0	98	KU854957
2	BJ-DEBCR-14	Bacillus subtilis	1615	99	0.0	99	KU854961
3	BJ-DEBCR-30	Bacillus subtilis	1177	99	0.0	99	MF487828
4	BJ-DEBCR-31	Bacillus subtilis	2383	100	0.0	99	MF487829
5	BJ-DEBCR-37	Bacillus subtilis	985	96	0.0	97	MF487835
6	BJ-DEBCR-39	Bacillus subtilis	1764	100	0.0	99	MF487837
7	BJ-DEBCR-5	Bacillus licheniformis	1945	99	0.0	99	KU301337
8	BJ-DEBCR-38	Bacillus licheniformis	1184	99	0.0	97	MF487836
9	BJ-DEBCR-32	Bacillus amyloliquefaciens	1070	100	0.0	99	MF487830
10	BJ-DEBCR-27	Staphylococcus spp.	1646	96	0.0	99	MF487825

Table 3
16S rRNA sequence based identification of microbes from Tsutuocie with GenBank accession numbers

Sl. No.	Isolates	Closest related microorganism	Max. score	Query (%)	E value	Similarity (%)	GenBank Accession No.
1	BJ-DEBCR-7	Bacillus subtilis	2135	98	0.0	98	KU301335
2	BJ-DEBCR-8	Bacillus subtilis	1591	99	0.0	99	KU854956
3	BJ-DEBCR-25	Bacillus subtilis	1212	100	0.0	99	MF487823
4	BJ-DEBCR-10	Bacillus licheniformis	1116	91	0.0	98	KU854958
5	BJ-DEBCR-12	Bacillus pumilis	1458	96	0.0	98	KU854960
6	BJ-DEBCR-34	Bacillus pumilis	1692	94	0.0	99	MF487832

DISCUSSION

Fermented food products have a long history and they form significant part of the diet of many indigenous communities in the developing world¹⁴. Nagaland has a rich diversity of indigenous fermented foods, which so far are least explored. Fermented food products in Nagaland are still prepared at household level and most of the cases, natural or spontaneous fermentation occurs resulting in a product of variable quality. Bio-preservation of leafy vegetables and fruits to extend the storage life and to enhance safety of foods using the natural microflora are popularly practiced in Nagaland. These products are mostly non-salted and are usually sun dried after the completion of the fermentation period. Mostly, the production of vegetable based fermented products involves the natural fermentation process with various lactic acid bacteria playing dominant role in imparting flavor, taste and aroma¹⁵. However, in the present study, the most dominant microorganism isolated from *Hungrij*, belonged to the members of the *Bacillus* species namely *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilis* and *Bacillus amyloliquefaciens*. *Bacillus* species are reported to have high survival rates, which may be the

reason of its presence in the food products even after post-fermentation treatment of the fermented product¹⁶. *Bacillus amyloliquefaciens* is also reported to be one of the most prevalent gram-positive aerobic spore-forming bacteria with the ability to synthesize polysaccharides and polypeptides¹⁷. *Bacillus* species are also reported to produce acids which may be the reason for the acidic nature of the food¹⁸. Bamboo shoots constitute a major component of traditional cuisine in most of the Asian countries. It forms a rich ecological niche which harbors a plethora of microorganisms¹⁹. In the present study, the most dominant microorganism isolated from *Rhujuk/Bastanga*, belonged to the members of the *Bacillus* species namely *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens*. Studies have also reported the presence of *Bacillus* species in other fermented bamboo shoot products^{20,21}. It was also reported that *Bacillus subtilis* showed the highest level of efficiency in accumulation of total phytosterol, which are precursors of many pharmaceutically active steroids^{20,22}. *Staphylococcus* species was also isolated which are usually considered undesirable when the count is greater than 10^2 cfu g⁻¹. Low numbers of these organisms is indicative of poor handling conditions whereas high counts are frequently associated with incidence of food poisoning²³. Most commercial

cucumber fermentations rely upon growth of the microorganisms that is naturally present on the surface of cucumbers¹⁵. Cucumbers are mostly fermented by adding salt or acetic acid to limit the growth of spoilage microorganisms²⁴. However, during the preparation of *Tsutuocie*, no salt is added but instead water is added. In the present study, the most dominant microorganism isolated from *Tsutuocie*, belonged to the members of the *Bacillus* species namely *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilis*. The high pH of the product may be one of the reasons for the occurrence of *Bacillus* species in dominance over other microorganisms in the final product, due to the ability of the microorganisms to hydrolyze protein into amino acids and ammonia²⁵. *Bacillus* species were found to be predominant microflora in these fermented products, indicating their versatility and easy excess in the manufacturing process but it is reported that they are not involved in spoilage instead some of the *Bacillus* species prevent fungal growth^{26,27}. In addition to lactic acid bacteria, it is known that *Bacillus* members possess probiotic activity^{28,29}. The absence of lactic acid bacteria in most of the fermented vegetables and fruit, probably owns to loss of survival during aging process³⁰. The results of this study is also in agreement with the generally accepted concept that traditional fermentations are dominated by a few microbial species that are selected during the course of fermentation because of good adaptation to the food matrix^{31,32}.

CONCLUSION

In Nagaland, fermented food products are still produced by spontaneous fermentation. Consequently, there is immense variation in the microorganism involved as well as sensory characteristics and quality of the fermented products. Thus, improvement of crude traditional methods by employing modern scientific technologies is the need of hour to upgrade the quality and production

REFERENCES

- Steinkraus KH. Fermentations in World Food Processing. Compr Rev Food Sci Food Saf. 2002;1(1):23–32. DOI:<http://dx.doi.org/10.1111/j.1541-4337.2002.tb00004.x>
- Papadimitriou K, Pot B, Tsakalidou E. How microbes adapt to a diversity of food niches. Curr Opin Food Sci. 2015;2:29–35. DOI:<http://dx.doi.org/10.1016/j.cofs.2015.01.001>
- Abegaz K. Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of borde, an Ethiopian cereal beverage. Afr J Biotech. 2007 June;6(12):1469-78. Available from: <https://www.ajol.info/index.php/ajb/article/download/57589/45969/>
- Tamang JP, Watanabe K, Holzapfel WH. Review: Diversity of Microorganisms in Global Fermented Foods and Beverages. Front Microbiol. 2016;7:377. DOI: <http://dx.doi.org/10.3389/fmicb.2016.00377>
- Tamang JP, Tamang B, Schillinger U, Franz CMAP, Gores M et al. Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. Int J Food Microbiol. 2005;105(3):347–56. DOI:<http://dx.doi.org/10.1016/j.ijfoodmicro.2005.04.024>
- Watanabe K, Fujimoto J, Tomii Y, Sasamoto M, Makino H et al. *Lactobacillus kisonensis* sp. nov., *Lactobacillus otakiensis* sp. nov., *Lactobacillus rapi* sp. nov. and *Lactobacillus sunkii* sp. nov., heterofermentative species isolated from sunki, a traditional Japanese pickle. Int J Syst Evol Microbiol. 2009;59(4):754–60. DOI: <http://dx.doi.org/10.1099/ijs.0.004689-0>
- Achi OK. The Upgrading of traditional fermented foods through biotechnology. Afr J Biotech. 2005 May;4:375-80. Available from : <https://www.ajol.info/index.php/ajb/article/download/15111/111351/>
- Ahaotu I, Anyogu A, Njoku OH, Odu NN, Sutherland JP et al. Molecular identification and safety of *Bacillus* species involved in the fermentation of African oil beans (*Pentaclethra macrophylla* Benth) for production of Ugba. Int J Food Microbiol. 2013;162(1):95–104.

of fermented products at commercial scale while keeping intact their unique natural flavor, taste and aroma. Knowledge of microbial diversity during spontaneous fermentations is useful, for designing relevant starter cultures for standardization, which may be used for upgrading this traditional technology to large-scale industrial production and marketing of these fermented food products. The entire process is therefore important, not only from an academic viewpoint, but also for the conservation of indigenous knowledge and technologies through the characterization and preservation of the microflora associated with the traditional fermented food products.

FUNDING/ACKNOWLEDGEMENT

Authors are thankful to Department of Biotechnology, Ministry of Science & Technology, Govt. of India, New Delhi for financial assistance through Institutional Biotech Hub to Prof. C. R. Deb vide order No. BT/22/NE/2011. The facilities used from UGC-SAP (DRS-III) Programme is duly acknowledged.

AUTHOR CONTRIBUTION STATEMENT

Both the authors contributed equally in the paper. Prof. Chitta Ranjan Deb conceptualized the work, designed the experiments, arranged the fund for the work, supervised the work and corrected the manuscript. While, Ms. Bendangnaro Jamir executed the work as a part of her Ph. D. work, drafted the paper and done the necessary correction suggested by Prof. Deb.

CONFLICTS OF INTEREST

Conflict of interest declared none.

- DOI:<http://dx.doi.org/10.1016/j.ijfoodmicro.2013.01.001>
9. Motarjemi Y. Impact of small scale fermentation technology on food safety in developing countries. *Int J Food Microbiol.* 2002;75(3):213–29. DOI:[http://dx.doi.org/10.1016/s0168-1605\(01\)00709-7](http://dx.doi.org/10.1016/s0168-1605(01)00709-7)
 10. Moore E, Arnscheidt A, Kruger A, Strompl C MM. Simplified protocols for the preparation of genomic DNA from bacterial cultures. In: Akkermans ADL, van Elsas JD de BF, editor. *Molecular Microbial Ecology Manual*. 2nd ed. Kluwer Academic Press; 1999. p. 10–3.
 11. Lechner S, Mayr R, Francis KP, Pruss BM, Kaplan T, Wiessner-Gunkel E, et al. *Bacillus weihenstephanensis* sp. nov. is a new psychrotolerant species of the *Bacillus cereus* group. *Int J Syst Bacteriol.* 1998;48(4):1373–82. DOI:<http://dx.doi.org/10.1099/00207713-48-4-1373>
 12. DJ. L. 16S / 23S rRNA sequencing. In: Stackebrandt E, Goodfellow M E, editor. *Nucleic Acid Techniques in Bacterial Systematics*. New York: Wiley & Sons; 1991. p. 115–175.
 13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol.* 1990;215(3):403–10. DOI:[http://dx.doi.org/10.1016/s0022-2836\(05\)80360-2](http://dx.doi.org/10.1016/s0022-2836(05)80360-2)
 14. Adeyemo SM, Onilude AA. Molecular identification of *Lactobacillus plantarum* isolated from fermenting cereals. *Intl J Biotech Mol Biol Res.* 2014 Dec;5(6):59-67. DOI:<https://doi.org/10.5897/IJBMBR2014.0184>
 15. Breidt F, Pérez-Díaz I, McFeeters RF, Lee CH. Fermented vegetables. In: Doyle MP, Buchanan RL, editors. *Food Microbiology: Fundamentals and Frontiers*. 4th ed. Washington, D.C: ASM Press; 2013. p. 841-855. DOI: 10.1128/9781555818463.ch33
 16. Ouoba LII, Thorsen L, Varnam AH. Enterotoxins and emetic toxins production by *Bacillus cereus* and other species of *Bacillus* isolated from Soumbala and Bikalga, African alkaline fermented food condiments. *Int J Food Microbiol.* 2008;124(3):224–30. DOI:<http://dx.doi.org/10.1016/j.ijfoodmicro.2008.03.026>
 17. Geng W, Cao M, Song C, Xie H, Liu L, et al. Complete Genome Sequence of *Bacillus amyloliquefaciens* LL3, Which Exhibits Glutamic Acid-Independent Production of Poly-γ-Glutamic Acid. *J Bacteriol.* 2011;193(13):3393–4. DOI:<http://dx.doi.org/10.1128/jb.05058-11>
 18. Yan Z, Zheng X-W, Chen J-Y, Han J-S, Han B-Z. Effect of different *Bacillus* strains on the profile of organic acids in a liquid culture of Daqu. *J Inst Brew.* 2013;119(1–2):78–83. DOI:<http://dx.doi.org/10.1002/jib.58>
 19. Tomar SK. Fermented Bamboo Shoots: A Riche Niche for Beneficial Microbes. *J Bacteriol Mycol Open Access.* 2016;2(4):1–8. DOI:<http://dx.doi.org/10.15406/jbmoa.2016.02.00030>
 20. Jeyaram K, Romi W, Singh TA, Devi AR, Devi SS. Bacterial species associated with traditional starter cultures used for fermented bamboo shoot production in Manipur state of India. *Int J Food Microbiol.* 2010;143(1–2):1–8. DOI:<http://dx.doi.org/10.1016/j.ijfoodmicro.2010.07.008>
 21. Tamang JP, Tamang N, Thapa S, Dewar S, Tamang B, et al. Microorganisms and Nutritional value of Ethnic fermented foods and alcoholic beverages of North East, India. *Indian J TradKnowl.* 2012 Jan;11(1):7-25. Available from :<http://nopr.niscair.res.in/bitstream/123456789/13415/1/IJTK%2011%281%29%207-25.pdf>
 22. Wu J, Zheng J, Xia X, Kan J. Purification and Structural Identification of Polysaccharides from Bamboo Shoots (*Dendrocalamus latiflorus*). *Int J Mol Sci.* 2015;16(12):15560–77. DOI:<http://dx.doi.org/10.3390/ijms160715560>
 23. Flint JA, Van Duynhoven YT, Angulo FJ, DeLong SM, Braun P, Kirk M, et al. Estimating the Burden of Acute Gastroenteritis, Foodborne Disease, and Pathogens Commonly Transmitted by Food: An International Review. *Clin Infect Dis.* 2005;41(5):698–704. DOI:<http://dx.doi.org/10.1086/432064>
 24. Gates K, Costilow RN. Factors Influencing Softening of Salt-Stock Pickles in Air-Purged Fermentations. *J Food Sci.* 1981;46(1):274–82. DOI:<http://dx.doi.org/10.1111/j.1365-2621.1981.tb14580.x>
 25. Parkouda C, Nielsen DS, Azokpota P, Ivette Irène Ouoba L, Amoa-Awua WK, Thorsen L, et al. The microbiology of alkaline-fermentation of indigenous seeds used as food condiments in Africa and Asia. *Crit Rev Microbiol.* 2009;35(2):139–56. DOI:<http://dx.doi.org/10.1080/10408410902793056>
 26. Tendulkar SR, Saikumari YK, Patel V, Raghotama S, Munshi TK, Balaram P, et al. Isolation, purification and characterization of an antifungal molecule produced by *Bacillus licheniformis* BC98, and its effect on phytopathogen *Magnaporthe grisea*. *J Appl Microbiol.* 2007;103(6):2331–9. DOI:<http://dx.doi.org/10.1111/j.1365-2672.2007.03501.x>
 27. Pérez-Díaz IM, Altuntas EG, Juneja VK. *Microbial Fermentation in Food Preservation* [Internet]. *Microbial Control and Food Preservation*. Springer New York; 2017. p. 281–98. DOI:http://dx.doi.org/10.1007/978-1-4939-7556-3_13
 28. Li K, Zheng T, Tian Y, Xi F, Yuan J, Zhang G, et al. Beneficial effects of *Bacillus licheniformis* on the intestinal microflora and immunity of the white shrimp, *Litopenaeus vannamei*. *Biotechnol Lett.* 2007;29(4):525–30. DOI: <http://dx.doi.org/10.1007/s10529-006-9291-4>
 29. Debaets L, Vaniwarden P, Meeus N, Schimmel H, Philipp W, Emons H. First certified reference materials for molecular fingerprinting of two approved probiotic *Bacillus* strains. *Int J Food Microbiol.* 2009;129(1):16–20. DOI:<http://dx.doi.org/10.1016/j.ijfoodmicro.2008.10.028>
 30. Lindgren S. Antagonistic activities of lactic acid

- bacteria in food and feed fermentations. FEMS Microbiol Lett. 1990;87(1–2):149–63.
DOI:[http://dx.doi.org/10.1016/0378-1097\(90\)90703-s](http://dx.doi.org/10.1016/0378-1097(90)90703-s)
31. Hounhouigan DJ, Nout MJR, Nago CM, Houben JH, Rombouts FM. Microbiological changes in maw during natural fermentation. World J Microbiol Biotechnol. 1994;10(4):410–3. DOI:<http://dx.doi.org/10.1007/bf00144462>
32. Halm M, Osei-Yaw A, Hayford A, Kpodo KA, Amoa-Awua WKA. Experiences with the use of a starter culture in the fermentation of maize for kenkey production in Ghana. World J Microbiol Biotechnol. 1996;12(5):531–6. DOI:<http://dx.doi.org/10.1007/bf00419468>