Indian Journal of Traditional Knowledge
Vol. 11 (1), January 2012, pp. 134-138

Bacterial diversity associated with *tungtap,* an ethnic traditionally fermented fish product of Meghalaya

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Received 03.05.11; revised 30.06.11

Fermentation process for preparing *tungtap*- a traditionally fermented fish product consumed by the ethnic tribes of Meghalaya, is carried out at room temperature for a period of 3-6 months under slightly acidic condition and the end product is consumed along with regular meals. Pre-salted fish *(Puntius* spp. or *Danio* spp.) is mixed with fish fats and packed in earthen pots and left for traditional fermentation for a period of six months. Bacterial diversity associated with this fermented fish product contained *Lactobacillus* sp., *Enterococcus faecalis*, *Enterococcus* sp., *Streptococcus* sp., *Micrococcus* sp., *Staphylococcus aureus, Staphylococcus saprophyticus*, spores forming *Bacillus* sp, *Bacillus subtilis*, *Bacillus cereus, Bacillus thuringiensis* and halophilic bacilli. Yeasts characterized from the samples belong to *Candida* and *Saccharomycopsis* species.

Keywords: Ethnic tribes of Meghalaya, Fermented fish product-*tungtap*, Traditional fermentation, Earthen pots, Lactic acid bacteria.

IPC Int. Cl.[8]: A23L 1/325, A23L 1/326, C12M, C12N, A23L 3/00, A21, A23

Tungtap is a popular fermented fish (*Puntius* spp. and/or *Danio* spp.) product, commonly prepared and consumed by the *Khasi* and *Jaintia* tribes of Meghalaya in North-Eastern state of India. This practice is similar to traditional processing of fish such as fermentation, salting, drying and smoking which are principal methods of fish preservation in South-East Asia[1]. A fermented fish can be described as any fishery product that has undergone degradative changes through enzymatic or microbiological activities either in the presence or absence of salt[2]. In the traditional method of preparation, fish is washed properly, dried in the sun for 3-4 days, supplemented with some amount of fish fats to create semi-anaerobic condition and kept in earthen pots. Earthen pots are stored at room temperature (18-28^{0}C) for 3-6 months until use. Proteolysis and liquefaction that occur during fish fermentation is largely due to autolytic breakdown of the fish tissues[3].

Preparation of *tungtap* is distinctively different from other fermented fish products such as *ngari* (*Puntius sophore*), *hentak* (*Esomus danricus*) of Manipur[4]. Some of the distinctive features of *tungtap* is that it does not fall under the category of fish paste or fish sauce[5] as it is consumed as pickle and taste enhancer. Although the fish product still retains the original shape, it gets softened due to pre-salting of fish prior to fermentation. The uniqueness of this process is that the fish is previously dried to very low water activity (a_W) which renders less favorable conditions for microbial growth[6, 7, 8]. Foodborne pathogenic bacteria are inhibited at a_W of 0.92 or less that is equivalent to 13% salt concentration (w/v)[9]. Microorganisms play an important role in the later stage of fish fermentation leading to the degradation of tissue proteins. This study deals with the traditional method of preparation of *tungtap* and the bacterial diversity especially the lactic acid bacteria (LAB) associated with it.

Methodology

Traditional method of preparation of *tungtap*

Fishes (*Puntius* spp. and *Danio* spp.) (Fig. 1) are collected from local rivers from southern parts of Meghalaya like the Dawki and Shella popularly known as Brahamaputra valley and Bangladesh. These are sundried for 3-4 days and then kept in jute bags. The fishes are salted in salt: fish ratio of 1:10 (Fig. 2). The dried fish in 30-40 kg batches are

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supplemented with some amount of fish fats and packed in earthen pots which are sealed using fish scales, mud, oil slurry or polythene sheets to make the earthen pots airtight (Fig.3). The earthen pots are then kept at room temperature (18-28°C) for 2-6 months. Once the fish fermentation process is complete, it is taken out and sold in the market (Fig. 4).

Physiological and microbiological analysis

The fresh fermented fish samples (*tungtap*) were collected in sterile containers from various regions of Meghalaya and were transported to the laboratory for analysis. The samples were homogenized in mortar and pestle, taking 10 gm of well mixed sample blended in 90 ml of 0.85% (w/v NaCl) physiological saline. The pH, temperature and water content of the samples were determined using standard methods. For isolation of LAB, the diluted inocula were plated out on deMan Rogosa and Sharpe (MRS) agar[10] and the plates were incubated at 37° C for 48-72 hrs. For enumeration of endospores forming bacteria, inoculum was plated in nutrient agar medium (HiMedia, India) and the plates were incubated at 30° C for 48 hrs. Total viable counts was determined after incubation at 30° C for 48 hrs by the plate count agar method[11]. Colonies were randomly picked from the plates and slant cultures were prepared on nutrient agar tubes for further analysis. The purity of the cultures was confirmed by extensive streaking on nutrient agar. Biochemical characterization included Gram staining, catalase, cytochrome oxidase, Hugh Leifson test, methyl red and Voges-Proskauer test, nitrate reduction, citrate utilization and indole production. The motility was checked besides other physiological and colony characteristics. LAB and endospore forming bacteria were identified by using standard methods[12,13].

Results and discussion

The ripening of *tungtap* takes 3-6 months that leads to softening in pre-salting fermentation procedure as compared to post-fermentation salting process. It developed slightly brownish pale colour with unique aroma once fermentation was complete compared to the fresh unfermented fish where such smell was not noticed. The microbial diversity constituted species of *Enterococcus*, homofermentative cocci (*Enterococci, Streptococci*), heterofermentative rods (*Lactobacillus*), endospore-forming rods (*Bacillus*), aerobic coccus (*Micrococcus* sp.) and yeasts (*Candida* sp., *Saccharomycopsis* sp.) along with pathogenic forms like *Bacillus cereus, Staphylococcus aureus* and *Clostridium* sp.

Enterococcus spp., *Streptococcus* spp., *Lactobacillus* spp. were observed with log CFU/gm of 4.8-5.5 in one day old sample which increased slightly to 4.8-5.8 in seven days old sample. There was no significant change in the density of endospore formers, total viable counts, moulds and yeasts with regard to one day and seven day old samples (Table 1). Aerobic mesophiles represented a high microbial population (log CFU/gm 7.4-9.0) as reported previously in *momone*, a fermented fish products in Ghana[14-16]. These comprised mostly *Micrococcus* sp. and *Bacillus* sp. with 60% and 40% respectively, of total bacterial flora in one week old ripened *tungtap*. The presence of low density of *Staphylococcus aureus, Clostridium* sp. and yeast contaminants in some of the fermented fish samples could be due to contamination and indicated that there is a need for improved handling and processing[15].

Tungtap is different from other fermented fish products such as those in Africa, where their popularity has been influenced by the fish consumption pattern[16], and reported to be relatively higher in the coastal countries due to proximity to the source of fish than in the inland[17] which is far from the source of fish. The distinct smell of this fermented fish has influence on consumer's orientation patterns and may not be palatable to everyone. However, in the absence of fresh fish, cured fish products such as smoked, salted, sundried and fermented fish are popular, which become a good source of animal proteins especially in rural areas. Preparation of this fermented fish product takes several months to develop a desired flavour during which the process encounters changes in population dynamics of LAB and other microbes necessary for fermentation. This kind of food fermentation allows bacteria, yeasts and molds to predigest, and therefore, break down the carbohydrates, fats, and proteins which carry bacteria into our digestive tract[18]. The presence of pathogenic *Bacillus cereus*, *Staphylococcus aureus* and members of Enterobacteriaceae is suggestive of microbial contamination during processing in a traditional fermentation process[19]. A small number of *Bacillus* spp. in foods is not considered significant[19]. *Staphylococcus* sp. is regarded as a poor competitor and its growth found in fermented foods is generally associated with a failure of the normal microflora[20].

Fig. 1 – Fresh fish *Puntius* sp.; Fig. 2 – Dried, salted and fat added *Puntius* sp.; Fig. 3 – Earthen pots where fishes are stuffed for fermentation; Fig. 4 – Ripened *tungtap* available in the market for sale

Table 1—Microbial counts and physico-chemical properties of *tungtap* (1 day & 7 days old) samples

No. of fermented samples collected from markets of Meghalaya

	pH	Temp (C)	Moisture (%)	Molds and yeasts			
				LAB	Endospore formers	Total viable counts	Molds and yeasts
Fifteen samples each							
Tungtap (one day old)	6.0 ± 0.1 - 6.2 ± 0.1	18.0 ± 4.6 – 26 ± 2.6	34.0 ± 1.0 - 36.2 ± 2.0	4.8 ± 0.2 - 5.5 ± 1.2	3.3 ± 0.0 - 5.0 ± 1.0	7.4 ± 1.2 - 9.0 ± 1.0	5.0 ± 0.0 - 5.6 ± 0.1
Tungtap (seven days old)	6.0 ± 0.1 - 6.3 ± 0.2	18.0 ± 4.6 – 26 ± 2.6	34.0 ± 3.0 - 36.2 ± 2.0	4.8 ± 0.3 - 5.8 ± 1.4	3.4 ± 0.1 - 5.0 ± 1.0	7.3 ± 0.8 - 8.9 ± 1.0	5.2 ± 0.3 - 5.7 ± 0.1

Values are ±SD given as range

Conclusion

In the traditional processes, due to lack of scientific investigation to standardize the methods, the use of poor quality raw materials, improper salting, inaccurate method of fermentation, water content, microbial contaminants and other unfavourable conditions, gradual deterioration of the quality of fermented fish takes place[21]. As regards to *tungtap*, there has been no proper traditional method of packaging this fermented fish product for transportation and effective commercialization because earthen pots are still used for fermentation and the product is marketed in jute sacks that are not very conducive for storage and transportation[22]. Glass bottles, plastics or more advanced modern techniques are not yet effectively used. Presently, cheaper plastic containers are replacing the traditional types. The most important function of packaging the fermented fish products is that the containers are air-tight to facilitate fermentation and storage.

The prevalence of LAB, which contribute to health-promoting benefits underlines the need and significance of scientific approaches in its production, storage and consumption. Investigations on their microbial enzymatic profiles may reveal their probiotic significance and importance of identification and development of starter cultures.

Acknowledgement

GF Rapsang acknowledges the DBT-JRF research fellowship received from Department of Biotechnology, Govt. of India to carry out the present study.

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