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# *Tungrymbai*- A traditional fermented soybean food of the ethnic tribes of Meghalaya

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*Tungrymbai* is a fermented indigenous soybean food, common to the ethnic tribes of Meghalaya. The microbial diversity in this food is studied to assess the nature of microbes and their source during spontaneous fermentation. The microorganisms associated with this fermented food are also present in the equipment and materials used during preparation and packing. Various species of lactic acid bacteria, yeasts and spore forming forms were recovered in the isolation process which included *Bacillus subtilis* (Ehrenberg), *Entercoccus faecium* (Orla-Jensen) Schleifer and Klipper-Balz, *Candida parapsilosis* (Ashford) Langeron and Talice, *Geotrichum candidum* Link, *Saccharomyces bayanus* Sacc. and *Saccharomycopsis fibuligera* (Linder) Klocker. The fermenting microbes were found to be associated with the equipments and materials used during the spontaneous fermentation of *Tungrymbai*. Spore forming forms were isolated from all the materials and equipments used during the process while lactic acid bacteria and yeasts could be isolated only from selective materials.

**Keywords**: *Tungrymbai,* Traditional food, Microbial diversity, Fermented food, Fermented soybean food, *Khasi*

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Microorganisms are naturally found in foods, which also include food such as fermented soybean. Fermented foods are typical of the region and exhibit unique flavour and texture that may not be palatable to everyone. Fermented products form an intrinsic part of the diet of the tribal people in Northeast India. *Tungrymbai* is a traditional fermented food product prepared from soybean seeds used in Meghalaya by the indigenous *Khasi* tribe. *Tungrymbai* is a popular fermented soybean based sticky food which serves as a cheap source of high protein food in local diet. Preparation and consumption of this food reflect deep rooted food culture of the ethnic communities. Preparation of *Tungrymbai* is exclusively practiced by people using indigenous technology. Soaked soybean seeds are cooked until they can be pressed easily. Excess water is drained off and placed in basket lined with locally grown fresh leaves of *Pyrnium pubinerve* Bl. (Marantaceae) locally called *slamet* and jute bags and left to ferment naturally in ambient temperature (25-40$^0$C) preferably near/over the fire place for 3-4 days. *Tungrymbai is* similar to *Kinema* of Bhutan, Nepal, Darjeeling and Sikkim. *Tungrymbai is* reported to be a good source of protein and other nutrients[1]. The study examined the materials and equipments such as raw soybean seeds, wooden grinder (mortar & pestle), wrapping leaves involved during the preparation of *Tungrymbai* to establish the source of fermenting microorganisms. It is well established that the process of fermentation enhances the nutritional quality of any product by enhancing the amount of vitamins and protein solubility. Knowledge of the sources of the microorganisms associated during natural fermentation of *Tungrymbai* would help to establish genetic resources of fermentation process. So far, microbiota associated with natural fermentation of *Tungrymbai* has not been assessed and hence it has been attempted to assess the microbiota of the fermented food and its sources.

## Methodology

Local variety of soybean seed [*Glycine max* (L.) Merrill] as well as *Tungrymbai* samples were purchased from local market [Bara Bazar (Iewduh)], aseptically kept in ice-box and transported to laboratory for analyses. Fresh leaves of *Pyrnium pubinerve* Bl. were also collected from villages where

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*Tungrymbai* is made. 10 gm well mixed sample was blended in 90 ml of 0.85% (w/v) sterile physiological saline in a lab-blender for 10 min. For aerobic endospore counts, 1 ml dilution was mixed with 9 ml sterile physiological saline, and heated for 2 min in continuously boiling water[2]. Decimal dilution series was prepared in sterile diluent and 1 ml of appropriate diluted suspension was mixed with molten media and poured into plates. Circular discs (0.5 cm diameter) of fresh leaves of *Pyrnium pubinerve* were cut with flamed sterilized cork borer and washed in sterile physiological saline for isolation of phylloplane microflora[3]. One ml of leaf wash was inoculated into plate containing nutrient agar (HiMedia M001) for enumeration of endospore forming bacteria and incubated at 37°C for 24 hrs. Lactic acid bacteria were enumerated on deMan, Rogosa and Sharpe(MRS) agar (HiMedia M641) supplemented with 1% calcium carbonate and incubated anaerobically at 30°C for 72 hrs. Total viable counts were determined using plate count agar (HiMedia M091A) after incubating at 30° C for 48 hrs. Wooden grinder (mortar & pestle) used during *Tungrymbai* preparation were rinsed with sterile distilled water, which was collected in sterile bottles, and transported to laboratory immediately for microbial analysis using dilution plate methods as described above Samples were examined for the presence of yeasts and moulds, using malt extract agar (HiMedia M924) supplemented with 100 $mgL^{-1}$ chloramphenicol and incubating anaerobically at 28°C for 72 hrs. Presence of Enterobacteriaceae was tested by using selective violet red bile glucose agar (HiMedia M581) and incubating at 30°C for 48 hrs. Colony, cell morphology and Gram staining comprised initial characterization of bacterial isolates. All other phenotypic characterizations of bacterial isolates were carr ied as per standard protocols[4,5]. Endospore forming bacteria were identified according to the keys[6]. Lactic acid bacteria were identified following the taxonomic keys[7]. Yeasts isolates were identified by the standard morphological and biochemical tests[8].

## Results and discussion

The microbial population in different sources available for the fermentation of *Tungrymbai* is tabulated (Fig. 1, Table 1). 48 strains of rod shaped, Gram positive, endospore forming bacteria were identified as *Bacillus subtilis* (Ehrenberg)[6]. Thirty strains of cocci-shaped, Gram- positive, non-spore forming lactic acid bacteria were identified as *Enterococcus faecium* (Orla-Jensen) Schleifer and Klipper-Balz[9]. Gas production from glucose was used as a first step in the differentiation of lactic rods[10]. Yeasts recovered from various sources were identified as *Candida parapsilosis* (Ashford) Langeron and Talice, *Saccharomyces bayanus* Sacc., *Saccharomycopsis fibuligera*(Linder) Klocker and *Geotrichum candidum* Link[8]. *Bacillus subtilis, Enterococcus faecium, Candida parapsilosis* and *Geotrichum candidum* were reported from fermentation of *Kinema*[11]. However, microbial analysis of raw soybean seeds showed the presence of *B. subtilis* spores. Besides *B. subtilis*, population of *E. faecium* and yeasts occurred predominantly in soaked soybeans, which is the starting material for *Tungrymbai* fermentation. This result indicates that the LAB and yeasts enter through water sources during the fermentation process. The wooden grinder revealed the presence of LAB, yeasts as well as spore formers thus supplementing the fermenting population of microbes during *Tungrymbai* preparation.

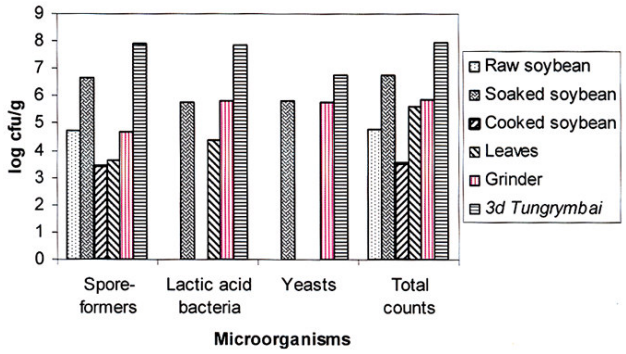


Fig. 1 — Microorganisms associated during fermentation of *Tungrymbai*

Table 1— Microbes found in various sources

| Microbes types | Raw soybean | Soaked soybean | Cooked soybean | Leaves of wrapping materials | Morter & pestle (Grinder) | 3 days old *Tungrymbai* |
|---|---|---|---|---|---|---|
| Spore former | $5.4 \times 10^4$ | $4.8 \times 10^6$ | $2.8 \times 10^3$ | $4.2 \times 10^3$ | $4.8 \times 10^4$ | $8.5 \times 10^7$ |
| Lactic acid bacteria | - | $6.3 \times 10^5$ | - | $2.4 \times 10^4$ | $6.8 \times 10^5$ | $7.6 \times 10^7$ |
| Yeasts | - | $6.7 \times 10^5$ | - | - | $5.7 \times 10^5$ | $5.6 \times 10^6$ |
| Total counts | $5.8 \times 10^4$ | $5.9 \times 10^6$ | $3.4 \times 10^3$ | $4.3 \times 10^5$ | $7.1 \times 10^5$ | $8.8 \times 10^7$ |

Fig. 2 Mortar & pestle, making of *Tungrymbai*

Fig. 3 *Tungrymbai* wrapped in leaves,

Fig. 4 Selling *Tungrymbai* in the market

Cells of microorganisms were killed during cooking of soaked soybeans except heat resistant spore formers. Phylloplane microbial analysis of fresh leaves of *Pyrnium pubinerve*, which is used as wrapping material for cooked *Tungrymbai* shows the presence of *B. subtilis* and *E. faecium* (Figs 2-4). Yeasts were not recovered from these leaves. There is report of presence of *Bacillus* and *Xanthomonas* sp in fig leaves (*Ficus hookeriana* Corner.) used as wrapping material during *hawaijar* production[12]. The population of yeasts as well as lactic acid bacteria increased remarkably in *Tungrymbai* fermented for three days, stored at room temperature. *B. subtilis* was found in all sources indicating its importance in *Tungrymbai* fermentation (Table 1). Sources like raw & soaked soybean seeds; leaves harness rich microbial diversity for spontaneous fermentation of *Tungrymbai*. Use of same grinder, which is not cleaned frequently to retain the microbiota, for grinding and same kind of leaves for packing supply microorganisms for spontaneous fermentation of *Tungrymbai* without using starter culture. *Bacillus* strains have been shown to possess strong peptidase and phosphatase activities during fermentation of *Kinema* thus increasing its nutritive value[13].

Knowledge of microbial diversity and their sources of the traditional indigenous fermented food like *Tungrymbai* will help in establishing their genetic resources and preparing their database. The development of starter culture will enhance the nutritive value of the product. Further studies on their enzyme profiles may reveal their importance as probiotics with relation to traditional foods used by the ethnic tribes.

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