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# A REVIEW ON THE POTENTIAL SCOPE TOWARDS INDUSTRIAL DEVELOPMENT AND CULTURAL CONSERVATION OF THE FERMENTED FISH PRODUCTS OF INDIA

**BIJAYALAKSHMI DEVI NONGMAITHEM*[1], AJIT KUMAR NGANGBAM[2], LAISHRAM LENIN[2], NITYANAIBEDYA BISWAL[3] AND NANAOCHA SHARMA[4]**

[1]Manipur University, Canchipur, Imphal 795 003, Manipur, India
[2]South Asian Institute of Rural and Agricultural Management (SAIRAM), Imphal 795 001, Manipur, India
[3]University of Algarve, Campus da Penha, 8005-139 Faro, 289 800100/900-Portugal
[4]Institute of Bio Resources and Sustainable Development (IBSD), Imphal 795 001, Manipur, India

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**Abstract–** Fermented food has been consumed as a delicacy across many regions along the globe. This review addresses the specific need of the metagenomics and transcriptomics approach in understanding the traditional fermented fish products of India, which uses the fish *Puntius* sp. as raw material. The fermented fish products play a very important role in the socioeconomic and livelihood specifically of the underdeveloped Northeastern states of India. There were reports on the presence of the healthy probiotic bacteria from the culture dependent microbial works. The isolated probiotic bacteria were reported to show antimicrobial spectrum against common pathogenic bacterial strains and has antioxidant properties. Till date, no work has been focussed on culture-independent techniques, establishment of safe starter culture and extension of shelf life of the fermented products of India. Such studies could potentially help to generate information towards a safety line of production for industrial development. This study also strongly raised the need for protecting the processes of traditional fermentation techniques that are employed by the respective place of origin. As the original ancestral techniques employed for the production of the individual fermented fish products has close resemblance, it is an urgent need to conserve these ethnic ancestral products, which will represent as a reference to demonstrate and witness the existence of rich culture of North-eastern India. Further, suggestions are being made on maintenance of production data bank, to support the verbal fact that these fermented fish product is an extremely important daily essential diet of the north-eastern India.

## INTRODUCTION

Fermented food is a broad term applied to the traditional ways of food preservation using the native knowledge from locally available plant or animal sources since ancient periods, which involves the effect of naturally evolved microorganisms. Food fermentation process generally targeted one of its constituent that caused significant modifications in physicochemical, sensory and enzymatic characteristics in the final products (Bevilacqua *et al*., 2016; El Sheikha, 2018). It is regarded as the best example of human innovation in the preparation of traditional food by using microbes, with no understanding of the underlying microbial flora involved (Katongole, 2008; Ray and Joshi, 2014; Chilton *et al*., 2015).

Fermented food serve about one-third of the world's population as a part of their Principal diet (Campbell-Platt, 1994). India is a vast country with vast natural resources that has close association with rich age-old culture and heritage. Food consumption, traditional processing methods and styles are very much related with the available resources and the choice of food habit of the people in the region. The excess produced were preserved traditionally based on the availability of the local raw materials and the preference of the taste of the locals. Traditional fermentation is one of the very important preservation techniques that are being employed globally and serve as delicacy of the places of origin. As such, among the various traditional food of India, fermented food and beverage products play a very important role in social and cultural practices of the region of India. There are more than 350 types of ethnic fermented foods and alcoholic beverages produced in India. Based on the source of raw materials, the fermented food could be classified as plant based or animal based. The meat and fish based fermented food of India are only popular and localised along the North eastern region of India (Tamang, 2020a) (Figure 1). Apart from Northeast India, traditionally preserve meat products are consumed in Jammu and Kashmir, Himachal Pradesh, Uttarakhand and Goa, without fermentation process, but are consumed as sun dried and smoked products (Tamang, 2020b). Similarly, there are also traditionally preserved fish products from other states of India, which are sun dried products from coastal region of Maharashtra, Goa, Tamil Nadu, Orissa and West Bengal. Among the fish based fermented products of India, the most commonly utilised raw material fish are the freshwater barbs *Puntius* sp. (Table 1). Despite of being the same raw material *Puntius* sp., the different fermentation processes have resulted into a drastic variation of the end products in terms of its biochemical profile and food microbiology.

Out of the 13 reported fermented fish products of India, five products are using *Puntius* sp. as a raw material (Table 1) (Figure 1). Out of these five,

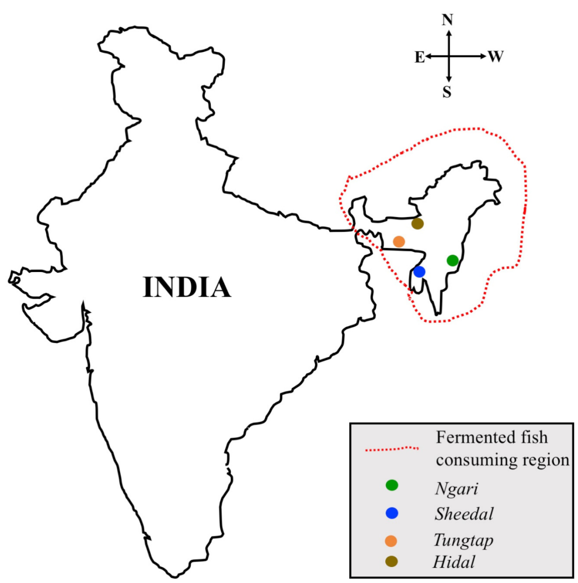


**Fig. 1.** Geographical indication of the fermented fish producing and consuming region of India.

*Numsing* of Assam is not only using *Puntius* sp. as raw material but it is a fermented paste of a mixture of freshwater fishes which includes *Puntius* spp., *Amblypharyngodon* sp., *Lepidocephalus* sp., *Channa* spp., *Trichogaster* spp., *Danio* spp., *Mastacembelus* spp., *Mystus* spp., *Rasbora* spp., etc. and petioles of *Alocasia macrorrhiza*. The other four are *Ngari* of Manipur, *Tungtap* of Meghalaya, *Hidal/Hidol* of Assam and *Shidal* or *Sheedal* of Tripura. However, *Hidal/Hidol* and *Shidal* or *Sheedal* are the same product with same process with different names. *Hidal/Hidol* are processed and prepared in Assam, whereas *Shidal* or *Sheedal* are processed in Tripura (Boruah *et al.*, 2017; Majumdar, 2020). Therefore, this exploratory work will specifically focus on the role of *Puntius* sp. on the industrial development based on its traditional uses as a basic raw material in the process of fermentation. The fermented products from *Puntius* sp.are as mentioned below:

1. "*Ngari*" fermented fish products of Manipur
2. "*Sheedal*" fermented fish products of Tripura
3. "*Tungtap*" fermented fish products of Meghalaya

### "*Ngari*" fermented fish products of Manipur

*Ngari* is a non-salted fermented fish product of Manipur prepared from sun-dried *Puntius sophore* (locally known as phabou) caught from streams and river and fermented in traditional pots known as Kharung (earthen pot). Apart from kharung, there is also another method of fermentation of *Puntius sophore* by using bamboo trunks and the product is known as *Utong-ngari*, but this method has become rare at present and is considered as tastier in comparison to *Ngari* which is fermented in kharung (Romi *et al.*, 2020). The use of fermented fish in the diet of the Manipuris are witnessed by the folktales of Manipur such as Hanuba Hanubi Pan Thaba, Houdong Lamboiba amasung Pebet, and Sanarembi Cheishra from the very ancient time since the existence of monarchical rule (Devi *et al.*, 2017).

*Ngari* is generally prepared during October to January as the raw materials are abundantly imported and locally available during this period. Phabou was traditionally employed in fermentation, as it is a low-cost fish and a bycatch fish. But with the growing demand and increase in population, its supply is now in shortage and hence being imported from Assam, Bangladesh, and Gujarat (Jeyaram *et al.*, 2009) for large scale production. The traditional method of *Ngari* preparation and its associated indigenous technical knowledge from many

**Table 1.** Fermented fish products of India.

| Sl. No. | State | Local name | Ingredient Source | Mode of uses | Fermented form | Reference |
|---|---|---|---|---|---|---|
| 1. | Arunachal Pradesh | *Ngiiyi-yaan* of Apatani Tribe | Freshwater fish | Consumed as chutney or pickle to enhance flavor in food | Whole fish fermented | Personal communication; Romi, *et al*., 2020, |
| 2. | Assam | *Hidal/Hidol* | *Puntius sophore* | Consumed as side dish | Whole fish fermented | Boruah, *et al*., 2017 |
| | | *Numsing* | *Puntius* sp., *Amblypharyngodon* sp., *Lepidocephalus* sp., *Channa* sp., *Trichogaster* sp., *Danio* spp., *Mastacembelus* sp., *Mystus* sp., *Rasbora* sp., and petioles of *Alocasia macrorrhiza* | Used in curry | Fermented Paste | |
| 3. | Manipur | *Ithiitongka* | Small freshwater fishes | Consumed as side dish | Whole fish fermented | Wanglar, *et al*., 2018 |
| | | *Ngari* | *Puntius sophore* | Used in curry and consumed as side dish | Whole fish fermented | Personal first hand information; Sarojnalini and Suchitra, 2009 |
| | | *Hentak* | *Esomus danricus*, petioles of *Alocasia macrorrhiza* | Used in curry and consumed as side dish | Fermented Paste | Personal first hand information, Romi, *et al*., 2020. |
| 4 | Tripura | *Lona Illis* | Indian shad (*Tenualosa ilisha*) | Consumed as side dish | Salted, chopped into pieces and fermented | Majumdar and Basu, 2010 |
| | | *Shidal* or *sheedal* | *Puntius* sp. | Used in curry and consumed as pickle | Whole fish fermented | Muzaddadi and Basu , 2012b; Majumdar, 2020 |
| 5 | Meghalaya | *Tungtap* | *Danio* sp. / *Puntius* sp. | Side dish and /or as pickle | Whole fish fermented; salted | Rapsang, *et al*., 2011; Rapsang and Joshi, 2012 |
| 6 | Mizoram | *Nghaum,* | Freshwater fish | Consumed as chutney and | Fermented paste mixed with vegetables | Personal communication; Uchoi, 2019 |
| | | *Nnghathu* | Freshwater fish | Consumed as chutney and mixed with vegetables | Whole fish fermented | Personal communication; Uchoi, 2019 |
| | | *Dan pui thu* | Mix freshwater fish and prawn | Consumed as chutney and mixed with vegetables | Whole fish fermented | Personal communication; Uchoi, 2019 |
| 7 | Nagaland | *Japangangnagtsu* | Freshwater Crab | Consumed as side dish | Whole fish fermented | Jamir and Rao, 1990 |

generations were documented and reported Jeyaram *et al.*, 2009; Romi *et al*., 2020; and Tamang, 2010) (Figure 2).

### '*Sheedal/ Shidal*' the fermented fish products of Tripura

*Sheedal* is a fermented fish product indigenous to Tripura, which are usually prepared from the small freshwater cyprinids fish *Puntius* sp. *Sheedal* are also locally known as, *viz. seedal*, *sepaa*, *hidal* and *verma*. Apart from Tripura, Assam also produce *Sheedal*, but Tripura is considered as one of the major producers of *Sheedal* along the Northeastern states. However, during the last two decades, the types of *Sheedal* available in Tripura were in two forms, namely *Punti Sheedal* and *Phasa Sheedal.* This is mainly due to the fact that, the retail price of dry or wet *Puntius* sp. were very high and in search for a low cost alternative, *Setipinna phasa* has been used as a dry fish for fermentation in place of *Puntius* spp. to produce low-cost *Sheedal* (known as *Phasa Sheedal*, *Telesch*, *Baspati Sheedal*, etc.). Due to the resulting two-fold lower cost of the end product from employing *Setipinna phasa* as raw material, *Phasa Sheedal* is popularly known as *Sheedal* for the poor (Majumdar, 2020). *Sheedal* is very popular in Tripura

Collection of fresh fish *Puntius sophore* and dried in the sun for 4-5 days.

↓

The sun-dried whole fishes are sorted out and removed unwanted dirts and broken fishes which are of lower quality.

↓

Washed the sundried fishes in plain water and kept soaking for 5-10min.

↓

The excess water are allowed to drain off by placing the washed sundried fishes in porous bamboo basket and left for overnight

↓

The drained washed fishes are spread on a cleaned polythene sheet or gunny bags.

↓

The spread fishes are further covered over with gunny bags and compressed over with legs or mechanical roller to drain off the excess water. The pressure applied also helps to break the spines of the fishes that flattens the fishes and imparting compact packaging for better fermentation process.

↓

Side by side the traditional earthen pot (Kharung) are smeared with mustard oil until completely saturated to establish anaerobic fermentation.

↓

The fishes are packed in traditional earthen pot (Kharung) coated with mustard oil in an airtight manner by pressing with legs or wooden rods.

↓

The mouth of the pot is sealed airtight with a thick paste mixture of trash fish, oil, sand and cow dung slurry.

↓

The packed and sealed earthen pot are allowed to stay in the room temperature for 6-12 months (fermentation process).

↓

End product- *NGARI*

**Fig. 2.** Traditional *Ngari* preparation process

and Assam among the Northeastern states of India. The origin of traditional *Sheedal* fermentation technology was believed to be very old before the partition of India and Bangladesh, i.e. before 1824 (Majumdar, 2020). The fermentation process of *Sheedal* was generally commenced during November to February as this was the harvesting period of the weed fishes once upon a time when there were huge shallow freshwater bodies. At present, the raw materials such as costly *Puntius* species and less costlier *Setipinna phasa* are imported from West Bengal, Gujarat, Uttar Pradesh and Bihar, with no specific production period. The step by step production process of *Sheedal* is listed in Figure 3 (Majumdar, 2020; Muzaddadi and Basu, 2012b). The end product '*Sheedal*' has a strong flavor and the visual appearance of good quality *Sheedal* consist of a whole fermented fish, compressed bilaterally with brownish glossy sticky surface.

**"*Tungtap*" fermented fish products of Meghalaya**

*Tungtap* is a popular fermented fish product commonly prepared from fishes such as *Puntius* sp. and *Danio* sp., consumed by the Khasi and Jaintia tribes of Meghalaya in the North-Eastern state of India (Joshi *et al.*, 2020). *Tungtap* is generally consumed as pickle/chutney along with cooked rice or boiled potatoes and as taste enhancer (Rapsang and Joshi, 2012). *Tungtap* are generally produced at the villages and marketed weekly at the local markets of Meghalaya (Sekar and Mariappan, 2007).

The raw materials for *Tungtap* preparation, i.e. *Puntius* spp. and *Danio* spp. are generally collected from local rivers like Dawki and Shella from the Southern parts of Meghalaya which is also popularly known as the Brahmaputra valley. The raw materials are also imported from Bangladesh. The traditional process of *Tungtap* preparation involves systematic procedure presented in Figure 3 (Joshi *et al.*, 2020; Rapsang and Joshi, 2012). The final steps of fermentation usually proceed by employing two different ways. The pre-fermentation salting and the post-fermentation salting. In post-fermentation salting, the method of preparation is

Descales and degut freshly collected fishes *Puntius* spp. or *Setipinna Phasa* and dried in the sun

↓

Washed with plain water.

↓

Allow to dry partially at room temperature for overnight.

↓

Traditional earthen pot (Matkas) are smeared with mustard oil until saturated.

↓

Airtight packing of the partially dried fishes in Matkas coated with mustard oil upto the neck

↓

The mouth of the packed earthen pots are sealing with a traditional paste (a thick paste made from ground old *Sheedal* and trash fish with oil and water) upto the thickness of 2–2.5 inches and covered with banana leaves.

↓

The packed and sealed earthen pot is kept for 3-4 days without any disturbance to observed the appearance of crack.

↓

The banana leaf that covers the sealed earthen pot are removed and applied with a thick layer of mud over and above the seal to fill up any airspace due to crack.

↓

Allow for fermentation until maturation (3–5 months) in a shade area or at room temperature

↓

End product- *SHEEDAL*

**Fig. 3.** Traditional *Sheedal* preparation process

similar to the pre-fermentation salting steps except that the salting of fish was done post fermentation prior to consumption or selling in the local retail market (Figure 3). In post fermentation, the fish develops a slightly brownish pale color with unique aroma compared to the sun dried fish prior to fermentation (Figure 4).

## Microbiological studies on Ngari, Sheedal and Tungtap

The fermented fish products of Manipur, *Ngari* are reported to have several probiotic bacteria (Angelin and Kavitha, 2020) and have antimicrobial (Khusro *et al*., 2020) and antioxidant activity (Singh, *et al*., 2018) (Table 2). Metabolites with antioxidant properties such as exopolysaccharides were isolated from the probiotic bacteria found in *Ngari* (Angelin and Kavitha 2020) (Table 2c). Bacteriocin from *Bacillus coagulans* isolated from *Ngari*, are reported to have the potential for its uses as a bio-preservative in food industries (Kaja *et al*., 2015) (Table 2c). The microbial flora isolated from *Ngari* were assessed for its biodiesel production, as the oleaginous yeasts isolated from *Ngari* form a potent source of microbial lipids which has a potential role in biodiesel production (Bardhan *et al*., 2020). The

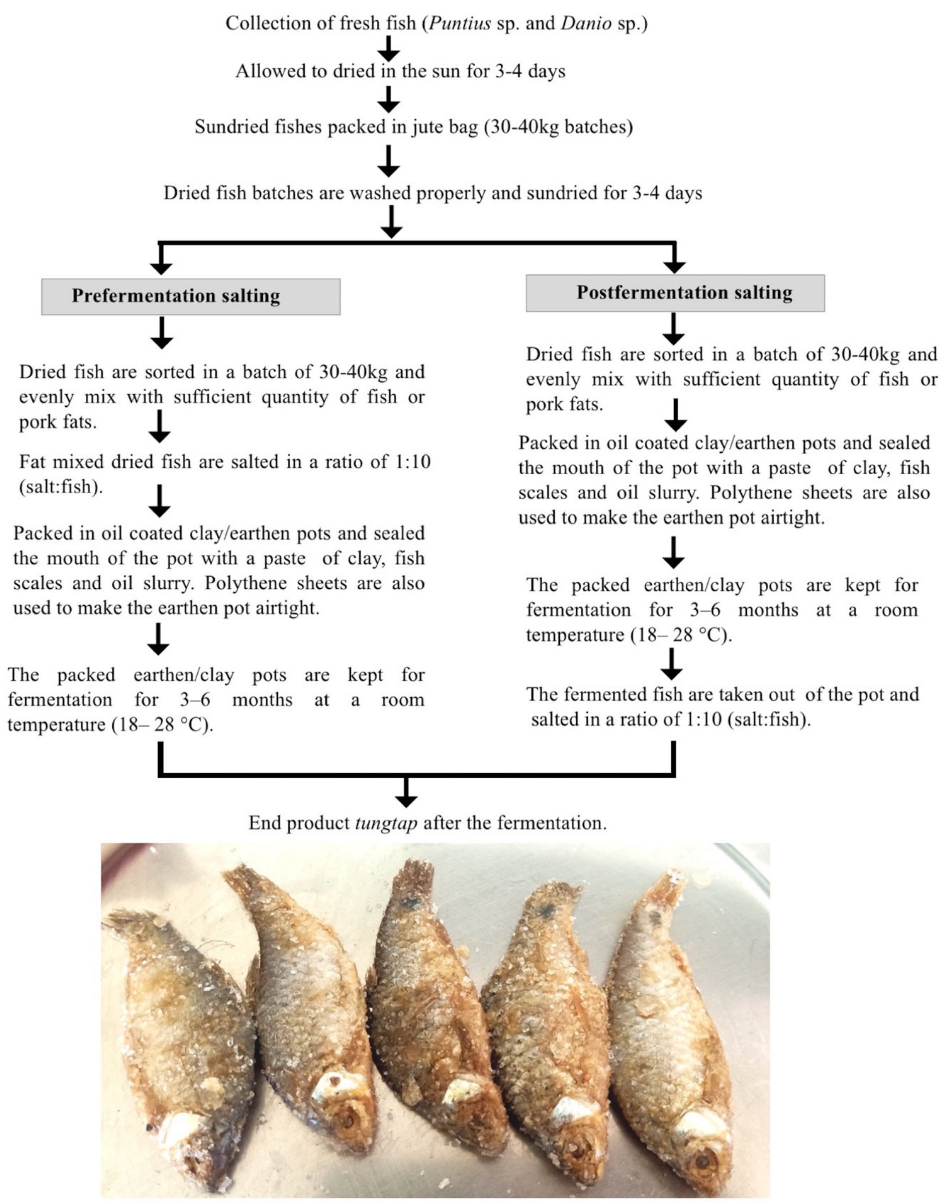


**Fig. 4.** Traditional processing methods of *Tungtap*.

**Table 2.** Microbial flora of *Ngari* A) Microbial count and B) Dominant bacteria and C) Bioactive metabolites of *Ngari* bacterial flora

**A)**

| Microbial parameters (cfu/g) | (Sarojnalini and Suchitra, 2009) | Log cfu/g (Thapa *et al*., 2004) | Log cfu/g (Majumder *et al*., 2015) |
|---|---|---|---|
| Total plate count of bacteria | 2.13 X $10^6$ | - | 6.65 ± 1.00 |
| Total Fungal count | 3.0 X $10^2$ | - | - |
| *Staphylococci* count | 2.66 X $10^6$ | - | - |
| Faecal *Streptococci* count | 6.33 X $10^3$ | - | - |
| LAB | - | 6.8 | 6.2 ± 0.08 |
| Bacterial endospores | - | 4.2 | - |
| Yeast | - | 3.1 | - |
| AMC | - | 7.0 | - |

**B)**

| Dominant bacteria | Source |
|---|---|
| *Bacillus coagulans, Bacillus pumilis, Bacillus. subtilis* and *Bacillus pantothenticus, Micrococcus* species such as faecal *Streptococci* and *Staphylococcus aureus.* | Sarojnalini and Suchitra, 2009; |
| | Sing *et al*., 2018 |
| *Staphylococcus cohnii subsp. cohnii, Tetragenococcus halophilus subsp. flandriensis,* a novel phylotype related to *Lactobacillus pobuzihii, Enterococcus faecium, Bacillus indicus,* Staphylococcus carnosus, Kocuria halotolerans, Clostridium irregulare, Azorhizobium caulinodans,* and *Macrococcus caseolyticus.* | Devi *et al*., 2015 |
| *Lactobacillus plantarum, Lactococcus plantarum, Bacillus subtilis, Bacillus pumilus, Lactococcus. lactis sub* sp. *cremoris, Enterococcusfaecium, Lactobacillus fructosus, Lactobacillusamylophilus, Lactobacilluscorynifomissub* sp. *torquens, Lactobacillus plantarum, Bacillus subtilis, Bacillus pumilus and Micrococcus sp.* | Thapa, *et al*., 2004 |

C) **Bioactive metabolites of *Ngari* bacterial flora**

| Bacterial flora in *Ngari* | Bioactivity | Metabolites |
|---|---|---|
| *Enterococcus faecium* (BDU7) | Probiotic strain (Abdul, *et al*, 2014) | Exopolysaccharide (Abdul, *et al*., 2014; Angelin and Kavitha, 2020) |
| | Strong DPPH and superoxide radical scavenging ability (*in-vitro*)(Abdul *et al*, 2014) | |
| *Staphylococcus saprophyticus* strain AAS1 | *In vitro* techno-functional properties (exhibited high tolerance at higher acidic conditions, simulated gastric juice of pH 2.0, and oxgall (0.5%, w/v), Strain AAS1 fermented varied carbohydrates and produced exopolysaccharide and lipase) (Khusro *et al*, 2020) | Exopolysaccharide and lipase. (Khusro, *et al*., 2020). |
| *Bacillus coagulans* | Bacteriocin shows antimicrobial activities toward a wide spectrum of food borne, and closely related pathogens with a MIC (Minimum Inhibitory Concentration) that ranged between 0.5 and 2.5 g/mL. This bacteriocin was reported as the smallest ever bacteriocin reported from *B. coagulans* with greater antimicrobial potency and lower cytotoxicity (Abdhul, *et al*., 2015) | Bacteriocin (Abdhul, *et al*., 2015) Shows the possibilities to be used as a bio-preservative in food industries. |

bacterial isolates from *Ngari* such as *Lactobacillus pobuzihi* and *Tetragenococcus halophilus* were reported to have anti-HIV activity at the laboratory scale (Imrat *et al*., 2020). The dominant microbial profile which are potential starter culture of *Ngari* was assessed by Sarojnalini and Suchitra (2009) (Table 2b). In the traditional fermentation process of *Ngari*, the fermentation process usually commences after 5 to 6 months of incubation whereas in case of the potential starter culture inoculated process, the fermentation process was reported to observe on 40 days after incubation (Sarojnalini and Suchitra, 2009). The bacteria and fungi grown during the process of fermentation of *Ngari* under different temperature conditions of 20 °C, 30 °C and 40 °C for every 30 days until six months was reported (Taorem and Sarojnalini, 2012). However, till date the microbial profile reported from *Ngari* are based on the traditional culture methods only.

The microbial profile of *Sheedal* are presented in Table 3 (Majumdar *et al*., 2015; Muzaddadi, 2015 and Muzaddadi and Mahanta, 2013). There are five natural stages for fermentation of *Sheedal* which takes 4-6 months that restricts to produce only two batches per year. Microbial floral at various stage of fermentation were assessed and reported. From stage three onwards i.e two and half months, there were no significant changes in the microbial flora (Muzaddadi, 2015), thereby shortening the harvesting time to produce greater number of batches per year. Further study to fasten the fermentation process by introducing known volume of potential starter culture with 100 ml bacterial inoculum ($10^8$ cell/ml) of *Staphylococcus aureus* and *Micrococcus varians* along with food additives such as 2% salt and 2% sugar in traditional method was reported (Muzaddadi and Basu, 2012a). This process helps in reducing the fermentation period to 45 days without losing the basic biochemical, microbiological and sensory characteristics of traditional best quality *Sheedal*. The *Staphylococcus* sp. isolated from *Sheedal* was reported to be sensitive to Chloramphenicol, Erythromycin, Norfloxacin, Co-Trimoxazole and Ofloxacin (Majumdar *et al*., 2015). Thus, the microbial studies in *Sheedal* till date is only restricted to the culturable microbes only.

The reports available on the microbial counts from *Tungtap* are presented in table 4a and the dominant microbes isolated from *Tungtap* are presented in Table 4b. Table 4 represents the culturable dominant microbial profile which consists of bacteria including Lactic Acid Bacteria (LAB) and pathogenic forms and yeast. Another study by Biswas *et al*., 2019, reported a co-occurrence of antimicrobial resistance and virulence determinants in enterococci isolated from *Tungtap*. The 38 enterococci isolated from *Tungtap* was identified as *Enterococcus faecalis* and out of the 38 isolates reported, 21% were able to hydrolyse gelatin and 34% showed protease activity. In a combined microbial study of *Tungtap*, a traditionally fermented *Puntius* sp. and *Tungrymbai*, a traditionally fermented soybean, a total of 84 isolates of LAB were reported (Biswa *et al*., 2017). Out of these 84 isolates, 11 potent bacteriocin-producing bacteria were isolated and reported as gram positive and catalase negative. Out of the 11 bacteriocinogenic LAB isolates reported, *Lactobacillus* was the most predominant bacteria which consisted of 72.7%. Further studies on LAB isolated from *Tungtap* was conducted by Rapsang *et al.*, 2011. The genomic DNA from the five selected LAB isolated from *Tungtap* was extracted and reported as *Lactobacillus pobuzihii* with ≥ 99.4%

**Table 3.** Microbial flora found in Puti *Sheedal*. A) Microbial parameter and B) Dominant bacteria

A)

| Microbial parameters | log cfu/g (Majumdar *et al.*, 2015) | log cfu/g (120 days of fermentation) (Muzaddadi, 2015) |
|---|---|---|
| Total plate count of bacteria | 6.87 ± 0.11 | 6.0 ± 0.2 |
| Total fungal Count | - | 2.4 ± 0.2 |
| Lactic acid bacteria (log cfu/g) | 4.5 ± 0.08 | - |

B)

| Dominant bacteria | Source |
|---|---|
| *Micrococcus spp., Staphylococcus aureus, Streptococcus/Enterococcus.,* | Muzaddadi and Mahanta, 2013 |
| *Bacillus spp. Escherichia coli, Staphylococcus spp.* | Majumdar *et al.*, 2015 |
| *Staphylococcus aureus* and *Micrococcus spp.* (at 120 days of fermentation) | Muzaddadi, 2015 |
| *Bacillus subtilis* and *Bacillus amyloliquefaciens,* | Sarifuddin *et al.*, 2015 |

similarity on the phylogenetic analyses of 16S rRNA gene sequences. Rapsang and Joshi, 2015 have reported another five LAB out of 50 isolates from *Tungtap* sample. The isolated LAB include *Lactobacillus pobuzihii, Lactobacillus pentosus, Lactobacillus rossiae* and *Lactobacillus plantarum*, which were reported as sensitive to commonly used antibiotics such as chloramphenicol, neomycin and erythromycin.

**Biochemical studies of Ngari, Sheedal and Tungtap**

The basic nutritional profile of '*Ngari*' was assessed along with certain quality parameters such as moisture (24.35 – 32.55%), crude protein (42.87 - 35.56%), total lipid (13.51- 15.53%), pH (6.14- 6.49), total volatile basic nitrogen (TVBN)(100.17- 173.33 mg%), thiobarbituric acid value (TBA)(0.42- 1.27 mg melonaldehyde $kg^{-1}$) and amino-nitrogen (10.28 mg $g^{-1}$) (Sarojnalini and Suchitra, 2009; Majumder *et al.* 2015 and Boruah *et al.*, 2017). The mineral compositions of *Ngari* was also reported by Majumder *et al*. (2015) which consist of Calcium, Potassium, Iron, Sodium, Manganese, Copper, Zinc and Magnesium. Further, fatty acid methyl esters (FAME) of the *Ngari* sample was also assessed and reported by Majumdar *et al*., (2015) which revealed that the most dominant fatty acid consist of oleic acid C18:1(n-9) with 23.58% followed by linoleic acid C18:2(n-6) with 11.68%. An unidentified fatty acid C16:1(n-5) with 11.36% was also reported from *Ngari*, which is almost parallel to the linoleic acid. The nutritional profile of the *Ngari* was also reported from the samples which were fermented at different temperature conditions of 20 °C, 30 °C and 40 °C for every 30 days throughout six months (Taorem and Sarojnalini, 2012). The effect of cooking on *in-vitro* digestibility and the antioxidant properties of *Ngari* was conducted by Hanjabam *et al.* (2020) and reported its positive health benefits. Antioxidant activity of exopolysaccharide from probiotic bacteria from *Ngari* was reported (Abdul *et al.*, 2014). The amino acid profile of *Ngari* reported by Majumder *et al*. (2015), shows that, the highest amino acid was consists of glycine 4.95 g/100 g dry weight. Future studies could be more focus towards the enzymatic roles in the fermentation process of *Ngari*.

There are various reports on the nutritional profile of *Sheedal* such as moisture (29.66- 38.26 %), crude protein (35.56- 36.84%), pH (5.7-6.23), alpha amino-nitrogen (16.75%), TVBN (62.53 mg%), peroxide value(PV) (9.53 milli eq./kg of oil), free fatty acid (6.48) and TBA(0.99 mg melonaldehyde $kg^{-1}$) (Majumdar *et al.*, 2016; Muzaddadi, 2015; Muzaddadi and Basu, 2012a and Boruah *et al*., 2017). The assessment of potential hazards for the presence of biogenic amines in puti *Sheedal* such as Putrescine, Cadaverine, Spermidine, Spermine and Histamine and the heavy metals such as Arsenic, Cadmium, Lead and Mercury lies within the safety consumption limit was reported by Uchoi, *et al.* (2018). In addition, fatty acid methyl ester (FAME)

**Table 4.** Microbial flora of Tungtap a) Microbial counts and b) dominant microbes

| a) Microbes isolated | c.f.u./g (Rapsang and Joshi, 2012) | log c.f.u. /g (Thapa *et al*., 2004) |
|---|---|---|
| LAB | 4.8± 0.2 - 5.5±1.2(*Enterococcus* spp. and 5.9 *Streptococcus* spp.) | |
| Endospore formers | 3.3±0.0 - 5.0±1.0 | 3.2 |
| Total viable counts | 7.4±1.2 - 9.0±1.0 | |
| Molds and yeasts | 5.0±0.0 - 5.6±0.1 | 3.0 |
| Aerobic mesophilic count (AMC) | | 6.1 |

| b) Dominant microbes | Source |
|---|---|
| **Bacteria:** *Enterococcus homofermentative cocc i*(*Enterococci*, *Streptococci*), *heterofermentative rods (Lactobacillus*), endospore-forming rods (*Bacillus*), aerobic coccus (*Micrococcus* sp.) | Rapsang and Joshi, 2012 |
| **Yeasts** *Candida* sp., *Saccharomycopsis* sp. | Thapa *et al*., 2004, Rapsang, *et al*., 2011, |
| **Pathogenic bacteria:** *Bacillus cereus,Bacillussubtilis, Staphylococcus aureus* and *Clostridium* sp., *Enterococcus faecalis.* Rapsang and Joshi, 2012, Thapa *et al*., 2004, Biswas, *et. al.* 2019 | |
| **Lactic Acid Bacteria:** *Lactobacillus coriniformis, Lactobacillus lactis, Lactobacillus fructosus, Lactobacillus pobuzihii, Lactobacillus pentosus, Lactobacillus rossiae* and *Lactobacillus plantarum*. | Rapsang and Joshi, 2015 |

analysis of *Sheedal* was reported by Majumdar, *et al*., 2015.An unidentified fatty acids C16:1(n-5) with 34.57% was the most dominant fatty acids reported from *Sheedal*. This particular fatty acids C16:1(n-5) was reported from *Ngari* as well (Majumdar *et al*., 2015). Among the known fatty acids, oleic acid C18:1(n-9) with 21.71% is the most dominant followed by DHA C22:6(n-3) with 15.3 %. An experiment to fasten the fermentation process was evaluated by studying the effects of salt, sugar and starter culture on fermentation biochemistry (Muzaddadi and Mahanta, 2013). This study reported the biochemical profiles (moisture, protein, ash, acid insoluble ash, total fat content, pH, free fatty acid, titratable acid level and non protein nitrogen (NPN) and sensory properties) of *Sheedal* and assessed by 10 judges in comparison to the best quality traditional *Sheedal*. Similar to *Ngari,* there have been no reports till date on the role of enzymes in the fermentation process of *Sheedal.*

In comparison to the reported studies on *Sheedal* and *Ngari*, lesser biochemistry related works have been reported from *Tungtap.* The basic biochemical profile of *Tungtap* reported from different sources consist of Moisture (34.00- 35.4 %), pH( 6.0-6.2), Dry Matter (66.5 / 100g), Protein (32.0- 40.6 g% on dry matter basis), Carbohydrate (37.1%), Fat (12- 19.6 g% on dry matter basis), Fiber (0.4 g% on dry matter basis) and Ash (32.2 g% on dry matter basis) (Rapsang and Joshi 2012; and Kakati and Goswami, 2013). Further, Kakati and Goswami (2013) have also reported the the minerals (Ca, Fe, Mg, Mn, Zn, P, Na, K) and vitamin contents (Carotene 29.4 and Retinol 12.6 IU %) from *Tungtap*.

**Potential areas to be addressed for future scientific studies**

**Establishment of the starter culture profile**

The scientific studies conducted so far on the ethnic fermented fish products of Northeast using *Puntius* sp. as basic raw materials were reviewed and presented above. It was found that, in all the three products *Ngari, Sheedal* and *Tungtap*, no reports are available as an established starter culture profile that could be readily used for industrial applications till date. The dominant microbes present in all the three fermented fish products were reported. In *Sheedal,* selected dominant bacteria with some additives like 2% salt, 2% sugar were found to employed and did the controlled fermentation and compared with best quality *Sheedal* (Muzaddadi and Basu, 2012a). Similar studies have not been reported from *Ngari* and *Tungtap.* These ancestral fermented fish products of India are consumed as a delicacy among the populations of the respective cultures. Therefore, it is no doubt safe to consume and has no visible health effect from the consumption of these fermented products and also are not reported. The microbial profile of these fermented products have further witnessed its health benefits by the presence of the probiotic bacteria and healthy metabolites such as bacteriocin with antimicrobial activity and exopolysaccharide. Nevertheless, there are also parallel reports on the presence of pathogenic microbes that might be due to unhygienic processing that warns the safety issue if not cooked properly. At this point, an example of the commercially established fermented products like greek yogurt could be cited where the fresh milk is pasteurized and known probiotic starter culture are inoculated in a sterile condition (Maragkoudakis *et al.,* 2006). This ensures the safety and healthy consumption for majority of the population including pregnant women. During pregnancy, it is recommended to incorporate and supplement with such healthy diet but with the traditional yogurt there are high chances of *Listeria* infection (Schaack and Marth, 1988) which is harmful for the newly developed foetus (Smith *et al.,* 2009). Such safety assurance could also be established in case of these fermented fish products with a strategic quality controlled and safety line of production. Therefore, establishment of the safe starter culture and safety line of production of the fermentation process is indispensable for these existing fermented ethnic products.

**Studies and profiling of the culture independent microbial flora**

The microbial studies reported so far from *Ngari, Sheedal* and *Tungtap* focussed only on the culture dependent methods (Table 2, 3 and 4). This implies the need to undertake culture independent methods to further investigate any unique symbionts or the most dominant symbionts that potentially play an important role in the fermentation process. Application of molecular techniques reveals more underlying information of the fermented products (El Sheikha and Hu, 2020). There are also reports on applications of molecular techniques in fermented fish products. DNA-sequencing of the fermented freshwater fish (*pla-ra*) of Thailand helped to investigate the microbial diversity in *pla-ra* revealing the dominant bacterial taxa in starter cultures

(Rodpai *et al.*, 2021). Metagenomic approaches such as pyrosequencing of 16S rRNA gene was applied on fermented salted fish *Narezushi* to investigate and analyze the microbial communities (Kiyohara *et al.*, 2012). The comparative study using culture-dependent methods and culture-independent methods to understand the ecology of lactic acid bacteria of "*adjuevan*", Ivorian fermented fish provides an understanding of the microbial biodiversity (Kouakou *et al.,* 2012). Application of molecular techniques in the fermented fish products of India may provide more underlying information on the processes of food fermentation. This will also improve the food quality, microbial role in enzymatic processes and understanding the source of flavor for industrial applications. Such intervention of technological knowhow will help in better understanding of the food processing technology where microbial symbionts play a major role.

**Potential areas to be addressed for cultural conservation**

Globalization has brought a busy and faster lifestyle and more accessibility to the products, goods and services (Scholte, 2008). Globalization also plays a very important role in evolving changes and fusion among the cultures and traditions across the globe. Therefore, it would not be wrong to state that the ethnic foods may also change its original aroma and texture which will eventually change the microbial and biochemical profile once the fusion processes occurs. Majority of the Southeast Asian countries have consumed fermented fish as a delicacy in their diet (Rattagool *et al.,* 1985). A very closely related fermented product of Malaysia, which uses *Puntius* sp. as raw material, is *Ikan pekasam* (Ezzat *et al.*, 2015) which is also consumed in Indonesia. The Malaysians also loved the taste of *Ngari* of India and revealed that is closely related with *Ikan pekasam* (personal observation of Bijayalakshmi Devi Nongmaithem). Therefore, there are high chances of fusion between the two products with the emerging cultural exchange and preference of taste between the populations. Another, very close example on *Ngari* itself is that, in Manipur, *Ngari* was initially evolved as *Utong Ngari* which is another method of fermentation of *Puntius sophore* by using bamboo trunks. But at present, this product was substituted by *Ngari* due to the influence of Hinduism and *Sheedal* fermentation (Romi *et al.,* 2020). As such, the original *Utong Ngari* are not at all observed in the present market (personal observation of Bijayalakshmi Devi Nongmaithem, Ajit Kumar Ngangbam and nanaocha Sharma). This might be due to the combined fact that the raw material bamboo required for fermentation are scarcely available and product output verses time consumed for processing might not be profitable. Overall, the profitability of the final output might be comparatively lesser due to which *Ngari* was opted over *Utong Ngari* by various producers despite of the fact that *Utong Ngari* is tastier. At present, if anybody would like to get access to the process of *Utong Ngari* fermentation, then the process has to be started from the documentation of old aged population who are related with such activities, which after a few generations might be in the state that information, cannot be recovered anymore. Therefore, to avoid such loss in the traditional ethnicity of a valuable food culture, it is very important to claim the intellectual property right on the processes. As microbes have a specific role in fermentation, it is also important to established the starter culture and claim IPR against it for the overall future industrial development of the place of origin.

**Existing market structure and potential areas to be developed**

At present, the production and marketing strategy for all the three fermented products remained scattered with no data available on the total production volume. There is also no documentation or reported information on the number of producers. Producers are randomly producing based on their adopted or ancestral skills. There is no organization that reports and monitors the quality and safety evaluation of these fermented products. Till date, there are only few studies on economic analysis of *Sheedal* and *Ngari* on the cost of production and cost benefit ratio from randomly selected producers (Upadhyay, 2016) and marketing cost analysis at three different channels (Geetarani and Churachand, 2019). It is a well-known fact that *Tungtap*, *Sheedal* and *Ngari,* are delicacies and serve as daily essential ingredients in the diet of the respective community. The raw material required, i.e. *Puntius* sp. is imported from Gujarat, West Bengal, Uttar Pradesh, Bihar and Bangladesh. Therefore, it is very important to establish the data for total production volume of these fermented fish products from each state that can possibly draw the attention of the policy makers to establish the needs for producing the raw materials within the states.

There are various ready to eat fermented shrimp paste imported in India along the Northeastern region from Myanmar with unknown labelling in their local script. People along the Northeast states prefer its taste and smell as it is much close to the fermented products available in the Northeastern region of India. As the imported product is processed and packed, it gives the sense of security to consume despite of its unknown labels. However, the fermented fish products of India remained localized till date and only the local consumer knows how to process and its associated risk. To increase the marketing options and popularize the fermented fish products, the information on the mode of consumption and its associated risk for these fermented products needs to be readily available globally which is only possible through the development of ready to eat, cooked and processed paste with control production ensuring the safety and quality controlled protocols. As such, research projects could also be focused on ready to eat control processing and packaging (for example hypothetical labels to consumers with certain attributes (Witkin *et al*., 2015) so that the data from this research and the developed products will be visible to the policy makers and concerned organizations / industrial agencies.

## CONCLUSION

There are various literatures and reports on the studies conducted on *Ngari* of Manipur, *Tungtap* of Meghalaya and *Shidal* or *Sheedal* of Tripura. Out of all the literatures and the research done so far in the last three decades on these fermented fish products, the major areas of studies consists of microbiology related activities and followed by biochemistry related works. The microbial works were focussed mainly on the isolation and characterization of dominant microbial flora from these fermented products. The microbial flora was assessed for their bioactive properties and their potential uses in industrial applications. The biochemical works were focussed mainly on the antioxidant properties in case of *Ngari* and *Sheedal* that arises due to the presence of bacterial flora. Studies were also made on nutritional profile, fatty acid profile, amino acid profile, effect of cooking, processing, organoleptic and sensory evaluation for *Ngari* and *Sheedal*. From the available literature, the potential areas for future studies are being addressed. Recommendations are being made to undertake further studies on culture-independent method, to claim the IPR against starter culture and ensuring safety line of production process of fermentation. Further, this study has suggested the need for a data bank on total production volume for each producing state to support the verbal fact that fermented fish products is a daily essential diet of the respective community. Also, the information on the mode of consumption and its associated risk for these fermented products needs to be readily available globally which is only possible through the development of ready to eat, cooked and processed paste with control production ensuring the safety and quality control protocols. It is the sole responsibility of the current generations to conserve the valuable age old culture and tradition of food before it loses its identity. At some point of time in future, these ethnic fermented ancestral food products will be the references to demonstrate and witness the existence of rich culture of the place of origin.

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**Conflict of Interest**

Authors show no conflict of interest.

Author(s) Contribution

B.D.N., N. A. K., L.L., N.B and N.S. designed the review; B.D.N., performed the literature search and wrote the paper., B.D.N., N.A.K and N.S. figure preparation; All authors commented and provided feedback on the manuscript.

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