Bacterial Composition in Shrimp Paste of Northern Sumatra Related to Flavor and Chemical Properties

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Abstract. Various bacterial species may be involved in the process of shrimp paste or Terasi fermentation which affects the flavor and aroma of the product. This study investigated the culturable bacterial isolates from each terasi with favored and unfavored taste along with its chemical properties. Four shrimp pastes were collected as representative samples from Northern Sumatra namely Asahan, Tanjung Pura, Tanjung Balai, and Langsa. The method of sample selection was purposive random sampling. The hedonic test was performed by 30 panelists to differentiate the favored and unfavored shrimp pastes. Bacteria were isolated from Terasi with the highest score for taste and aroma using Nutrient Agar medium + 10% NaCl and several cultures were heated at 80°C for 15 min for isolated Bacillus spp. The shrimp pastes were tested for their chemical characteristics in percentage (%) e.g. water content, carbohydrate, total fat, protein and salt content (NaCl). The hedonic test results showed that the shrimp paste originated from Langsa (Aceh) produced the highest score for taste (80.6%), aroma (81.1%) and the aroma of fresh shrimp paste (71.4%). The chemical characteristics of Northern Sumatra shrimp paste were, water content ranging from 29-34%, carbohydrate 0.89-2.91%, total fat 1.97-2.39%, protein 25-38%, and salt content 9.83-24%. Seven bacterial isolates were obtained from Langsa shrimp paste coded as IW1, IW2, IW3 and IW4 and three suspected Bacillus coded as B1, B2, and B3.

1. Introduction

Terasi is a term for Indonesian fermented shrimp paste products and its products are popular in Southeast Asia as a spice in cooking. Terasi belongs to fermented food because, during the manufacturing process, there is enough time for indigenous microbes to grow and carry out the fermentation process. Besides shrimp paste, other terms for this type of food are also found in other countries, for example Bagoong or Alamang (Philippines), Ngapi (Myanmar), Nappi (Bangladesh), Belachan (Malaysia, Brunei Darussalam), Ka-pi (Thailand, Cambodia), Shajiang (China), Mamruoc or Mamtom (Vietnam) and Saewoojeot (Korea) [1,2].

Research on the diversity of indigenous microbial communities originating from Terasi has been widely reported. The method used in revealing the diversity of microbial communities from shrimp paste has been reported from the use of conventional to modern/molecular techniques, especially bacteria with proteolytic abilities. Halophilic lactic acid bacteria from Terasi of Cirebon, West Java had cell densities of $10^4$-$10^6$ CFU.g$^{-1}$ on MRS media. Further investigation showed the identity of the Tetragenococcus species based on T-RFLP markers and 16s rRNA [3]. Thermophilic and mesophilic bacterial communities from Central Java Terasi was reportedly dominated by genera: Sulfitobacillus,
Vibrio, Alcaligenes, Aeromonas, Pseudomonas, Kurthia, Caryophanon, Amphibacillus and Bacillus based on biochemical characters [4]. The dominance of Bacillus species as indigenous bacteria was again reported from Terasi of West and Central Java which has potential proteolytic properties [5]. The diversity of Bacillus from various shrimp paste products has been reported based on literature searches and bioinformatics studies [6]. Based on the findings, the existence of indigenous bacteria from shrimp paste from various sources are spatially and temporally limited so that the possibility of finding new species or even communities of indigenous bacteria is relatively possible.

Through conventional methods, indigenous microbes from Terasi have been reported to have potential in producing various functional metabolites, for example, lactic acid, enzymes, and bacteriocin with the prospect of utilization in each applied field [3, 7, 8]. To date, research related to the diversity of proteolytic bacteria based on the identification of 16s rRNA to discuss the functional groups of bacteria in local fermented shrimp paste, especially in Sumatra, is still limited. By seeing the potential of indigenous microbes from shrimp paste from various countries and provinces in Indonesia, we assumed the presence of specific bacterial species suspected to contribute to the taste and aroma that characterize local Terasi from Sumatra.

2. Materials and Methods

2.1. Organoleptic analysis
Four samples of local terasi from Sumatra was collected as representative samples by exploring shrimp paste products from Aceh to Lampung province through information on traditional traders. Organoleptic testing of the local terasi was performed by 30 panelists. The hedonic test was based on quality standards and assessments suggested in the form of score sheets refer to Indonesian National Standard or SNI 01-2716-2009.

2.2. Proximate analysis
Water content, total fat, protein, and crude carbohydrates in percentage (%) from each local Terasi were examined according to the standards of The Association of Analytical Chemistry (AOAC), according to Indonesian National Standard for food analysis or SNI 01-2891-1992.

2.3. Salt content measurement
A total of 0.5–1.0 g of local terasi sample was mixed with 10–20 mL of 0.1 N AgNO3 and 10 mL of HNO3(p). The mixture was boiled for 10 min and cooled in running water. The mixture was filtered using Whatman filter paper No. 1. The filtrate was diluted to 50 ml with distilled water and 5 ml of the NH4Fe(SO4)2•12H2O indicator. The mixture is titrated with a standard 0.1 N KCN until the solution turns brownish-red. Salt content is measured and expressed in % NaCl according to SNI 01-2891-1992.

2.4. Water content analysis
A total of 1–2 g of local terasi sample was stored in vials with known mass (g) and dried in an oven at 105°C for 3 hr. The samples were dried in desiccator until constant weight. Moisture or water content expressed in % were measured before and after the drying process.

2.5. Total plate count of indigenous bacteria
A total of 10 g of local terasi sample was suspended in 90 mL peptone 0.1% and homogenized in stomacher. A total of 1 mL was diluted to 10⁻² with 0.85% NaCl, and spread on Plate Count Agar (PCA) media for general bacterial groups and on de Man, Rogosa, and Sharpe Agar (MRSA) media for the lactic acid bacteria group. For Bacillus spp., the suspension was heated at 80 °C for 15 min, prior plating on Nutrient Agar (NA) media. The cultures were incubated at ambient temperature for 2-7 days. Growing colonies were counted in expression of Colony-forming Units (CFU/g) [9] and purified as bacterial isolates.
2.6. Qualitative screening of proteolytic and chitinolytic bacteria

Pure bacterial isolates from NA plates were taken using sterile toothpicks and then spot on Casein Agar (CA) and Colloidal Chitin Agar (CCA) medium. After incubation at ambient temperature for 24-48 h, the diameter of the clear zone formed was measured using digital caliper. Proteolytic and Chitinolytic index were calculated as follows:

\[
\text{Chitinolytic index} = \frac{\text{Diameter of hydrolysis zone} - \text{Diameter of colony}}{\text{Diameter of colony}}
\]

3. Results and Discussion

3.1. Hedonic rating score of Northern Sumatran Terasi

Based on market surveys, there are 4 terasi samples based on the highest demands on the market or widely consumed in Northern Sumatra, namely Asahan, Tanjung Pura, Tanjung Balai, and Langsa region. A hedonic or preference test was performed on four Terasi samples using 30 panelists as consumers. Previously, Terasi was processed with Indonesian chili sauces and consumed with white rice during test. Langsa Terasi received the highest score for taste and aroma with a value of 80% and 81%. Whereas, terasi Asahan was scored the lowest 66.6% (flavor) although considered high in chili sauce for 81.1% (aroma) and 71.4% for the fresh Terasi aroma (Table 1). The taste and aroma of shrimp or fish paste can be influenced by the type and characteristics of the raw materials, the processing process and the types of microbes involved. In general, the Indonesian shrimp paste or terasi is made through accidental or natural fermentation which involves the microbes from environment.

### Table 1. Results of hedonic test of Northern Sumatran Terasi

| No. | Origin of sample | Taste | Aroma\(^a\) | Aroma\(^b\) |
|-----|------------------|-------|-------------|-------------|
| 1   | Asahan           | 66.60 | 81.10       | 66.10       |
| 2   | Tanjung Pura     | 70.50 | 72.40       | 49.20       |
| 3   | Tanjung Balai    | 78.20 | 79.20       | 73.40       |
| 4   | Langsa           | 80.60 | 81.10       | 71.40       |

Note: Aroma\(^a\) = Terasi prepared in chili sauce, Aroma\(^b\) = Fresh Terasi

3.2. Chemical composition of Northern Sumatran terasi

The highest water content was obtained from Tanjung Balai terasi with the percentage of 34.7%, followed by Langsa, Asahan, and Tanjung Pura with percentage of 34.10, 33.6, and 29.7%, respectively (Table 2). Our finding was similar to previous report on water content of Kapi, the Thailand shrimp paste with the content ranged from 33.79 to 52.4\[10\]. Water content may affect the texture and indirectly affect the taste and aroma of Terasi by structuring indigenous bacterial communities. However, the texture of Terasi is not discussed in this study. The highest carbohydrate content was obtained from Asahan Terasi with the percentage of 2.91%, being the less favored Terasi in our study. Carbohydrate content was considered as minor contributor to Terasi flavour, with only characteristics as texture enhancer.

### Table 2. Chemical composition of Northern Sumatran terasi

| No. | Origin of sample | Water content | Total Carbohydrate | Total Lipid | Total Protein | NaCl |
|-----|------------------|---------------|-------------------|-------------|--------------|------|
| 1   | Asahan           | 33.60         | 2.91              | 2.39        | 28.10        | 9.83 |
| 2   | Tanjung Pura     | 29.70         | 0.89              | 2.43        | 38.00        | 10.90|
| 3   | Tanjung Balai    | 34.70         | 0.90              | 2.48        | 37.00        | 11.50|
| 4   | Langsa           | 34.10         | 1.70              | 1.97        | 25.80        | 24.00|
Total protein content was obtained from Tanjung Pura terasi, with the percentage of 38%, yet perceived as less favored terasi by the panelists. In contrary, the total protein in Langsa terasi with the lowest total protein content (25.8%), received the highest score in taste by the panelists. Again, Kapi, also revealed the dynamic protein content ranging from 29.44 to 53.27%, compared to Northern Sumatran terasi with the highest content of 38%. The contribution of different shrimp species and other mixtures may contribute to different protein composition of shrimp pastes. In addition, we may see that shrimp paste as a good source of protein. Regarding the salt (NaCl) content, the highest percentage was obtained from Langsa terasi with 24%, while the others only ranges from 9–11.5%. NaCl content might be the limiting factor to indigenous bacteria, especially favoring the growth condition of halophilic bacteria. Meanwhile, Langsa terasi had the lowest fat content (1.97%) than other samples which assumed due to different type of raw material and the volume used.

3.3. Characteristics of bacterial isolates from Northern Sumatra Terasi

Seven bacterial isolates were successfully recovered from the most and least favored Terasi samples, i.e Langsa and Asahan. All isolates were given a code while molecular identification is currently on progress (Table 3). The number of halophilic bacterial population in this study was \(3.8 \times 10^4\) CFU/g which was slightly different from previous study on Cirebon, the Java Terasi around \(10^5\) CFU/g [2]. Different chemical composition and natural ingredients in Northern Sumatran Terasi may alter the bacterial composition in performing their optimum growth. We also found 3 isolates indicating species of Bacillus spp., from both Terasi, the most favoured terasi and the least one. Bacillus spp. are potential extracellular enzyme production such as protease and amylase, while attempt to recover lactic acid bacteria have been in progress. Occurrence of lactic acid bacteria is considerably high with population of \(10^4\)–\(10^5\) CFU/g in MRSA medium supplemented with 10% NaCl from Cirebon terasi [3]. Meanwhile, occurrence of LAB in other Northern Sumatra fermented food is still limited, especially from the indigenous food which may be evaluated progressively in the future [11].

Table 3. Colony morphological characteristics of bacterial isolates from Northern Sumatran Terasi

| No. | Origin of sample | Isolate code | Color        | Form        | Edge          | Shape | Gram |
|-----|------------------|--------------|--------------|-------------|---------------|-------|------|
| 1.  | Langsa\(^a\)    | IW1          | Milky white  | Irregular   | Entire        | Coccus| +    |
| 2.  | Langsa\(^a\)    | IW2          | Yellowish white | Circular   | Raised        | Rod   | -    |
| 3.  | Langsa\(^a\)    | IW3          | Yellow       | Circular    | Entire        | Coccus| -    |
| 4.  | Langsa\(^a\)    | IW4          | Cream        | Irregular   | Undulate      | Coccus| -    |
| 5.  | Langsa\(^a\)    | B1           | Yellow       | Circular    | Entire        | Rod   | +    |
| 6.  | Langsa\(^a\)    | B2           | White        | Circular    | Entire        | Rod   | +    |
| 7.  | Langsa\(^a\)    | B3           | Yellow       | Circular    | Undulate      | Rod   | +    |
| 8.  | Asahan\(^b\)    | IW16         | Yellowish white | Circular   | Entire        | Rod   | -    |
| 9.  | Asahan\(^b\)    | IW17         | Cream        | Circular    | Entire        | Coccus| -    |
| 10. | Asahan\(^b\)    | IW18         | White        | Circular    | Undulate      | Coccus| +    |
| 11. | Asahan\(^b\)    | A1B          | Yellow       | Circular    | Entire        | Rod   | +    |
| 12. | Asahan\(^b\)    | A2B          | White        | Circular    | Undulate      | Rod   | +    |

Note: Langsa\(^a\) = the most favored, Asahan\(^b\) = the least favored

3.4. Proteolytic and chitinolytic activities of bacterial isolates from Northern Sumatran terasi

Some bacterial isolates showed different abilities in producing proteolytic and chitinolytic activities (Table 4). The main ingredient of Indonesian terasi is small shrimp or rebon with minute composition of fish. Chitin is polymer compounds synthesized by shrimp as raw material in terasi. Approximately 40% of shrimp by-product contained chitinous material, encrusted with CaCO\(_3\) and astaxanthin [12]. Therefore, occurrence of proteolytic and chitinolytic microbes are essential during the fermentation.
process. Proteolytic or chitinolytic microbes could be present during Terasi fermentation processing. Microbial community in Indonesian terasi may be structured by bacteria, mold and yeast [13]. The highest proteolytic and chitinolytic activities was produced by isolate IW1 with diameter of hydrolytic/clear zone of 23.5 and 22.4 mm, respectively. Other bacterial isolates from Asahan, were observed to only exhibit proteolytic activities with no traceable chitinolytic activities. The output in Terasi fermentation process is secondary metabolites, mostly known as glutamate which contribute the umami flavor to Terasi. However, the main functional group or lactic acid bacteria were the primary producer of the compounds [8]. Future investigation may reveal the contribution of LABs and Bacillus group in bioprospecting the secondary metabolites produced by those microbes.

Table 4. Proteolytic and chitinolytic activities of bacterial isolates from Northern Sumatra Terasi

| No. | Origin of sample | Isolate code | Diameter of hydrolysis zone (mm) | 
|-----|-----------------|--------------|---------------------------------| 
|     |                 |              | Proteolytic | Chitinolytic |
| 1.  | Langsa          | IW1          | 23.50       | 22.40       |
| 2.  | Langsa          | IW2          | -           | -           |
| 3.  | Langsa          | IW3          | -           | -           |
| 4.  | Langsa          | IW4          | 10.60       | 23.30       |
| 5.  | Asahan          | B1           | NE          | NE          |
| 6.  | Asahan          | B2           | NE          | NE          |
| 7.  | Asahan          | B3           | NE          | NE          |
| 8.  | Asahan          | IW16         | 17.80       | -           |
| 9.  | Asahan          | IW17         | -           | -           |
| 10. | Asahan          | IW18         | 6.20        | -           |
| 11. | Asahan          | A1B          | NE          | NE          |
| 12. | Asahan          | A2B          | NE          | NE          |

Note: NE = Not evaluated

4. Conclusions
Based on hedonic rating score Langsa Terasi was the most favourable while Asahan was unfavourable one. Langsa Terasi has the highest salt contain compared to other Terasi while other chemical composition relatively was not much different. Sevent bacteria isolates were found in Northern Sumatra Terasi such as Gram positive coccus, Gram negative coccus and rod as well as Bacillus group. Lactic acid bacteria were also found but we still working in its characterization.

Acknowledgement
This research is fully funded by Universitas Sumatera Utara through scheme of TALENTA USU year 2019 with contract number: 4167/UN5.1.R/PPM/2019 on April 1st, 2019 to IJ.

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