

Characterization of Lactic Acid Bacteria (LAB) isolated from Indonesian shrimp paste (terasi)

U Amalia^{1*}, Sumardianto¹, and T W Agustini¹

¹Department of Fish Product Technology, Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 50275, Indonesia

Email : ulfah_amalia@yahoo.com

Abstract : Shrimp paste was one of fermented products, popular as a taste enhancer in many dishes. The processing of shrimp paste was natural fermentation, depends on shrimp it self and the presence of salt. The salt inhibits the growth of undesirable microorganism and allows the salt-tolerant lactic acid bacteria (LAB) to ferment the protein source to lactic acids. The objectives of this study were to characterize LAB isolated from Indonesian shrimp paste or "Terasi" with different times of fermentation (30, 60 and 90 days). Vitech analysis showed that there were four strains of the microorganism referred to as lactic acid bacteria (named: LABS1, LABS2, LABS3 and LABS4) with 95% sequence similarity. On the basis of biochemical, four isolates represented *Lactobacillus*, which the name *Lactobacillus plantarum* is proposed. *L.plantarum* was play role in resulting secondary metabolites, which gave umami flavor in shrimp paste.

Keywords: Fermentation, Lactic acid bacteria, Salt, Shrimp paste

1. Introduction

Shrimp paste was one of food fermentation, popular as a taste enhancer in many dishes in Indonesia, made by naturally fermentation between shrimp and salt. This product is made from salted small shrimps through a complicated process of grounding, drying, and solidifying [1]. A fermented shrimp paste (terasi), produced in Central Java, Indonesia, contains many compounds of macro and micro-nutrient which permits the growth of various micro-organisms, one of them is bacteria. The presence of bacteria in shrimp paste product could produce the secondary metabolites to give a specific taste and aroma of shrimp paste, which named *umami* taste [2]. However, there were less information about the secondary metabolites resulted from fermentation process, especially related to the microorganism fermenter.

The intensity of umami taste in food is usually produced throughout synergistic interactions of free glutamate and 5'-ribonucleotides [3]. In shrimp paste, glutamate came through fermentation process, degradation of protein source of shrimp. Numerous studies on the source of umami taste on shrimp paste were previously performed from various scientific [4]. In previous studies, Kaewklom et al. [5] found *Bacillus amyloquefaciens* from shrimp paste *kapi*, traditional ingredient in Thailand. Tanasupawat and Visessanguan [6] stated that



the bacteria isolated from fermented food are widely known to be safe for human consumption for their safety to human health. Similarly, Sumardianto et al. [7] NaCl and time of fermentation affected glutamate content of shrimp paste. The aim of this study was to characterize the physiological and biochemical of lactic acid bacteria isolated from shrimp paste (terasi).

2. Methods

2.1. Shrimp paste samples

Samples of 9 types of *terasi* used in the study were obtained from previous research that conducted 8 months before. The samples were *terasi* with time of fermentation for 30 days, 60 days and 90 days, and all conducted for 3 times repeated.

2.2. Isolated preparation [8]

The lactic acid bacteria characterized in this study were isolated from shrimp paste samples. The striking out-plate technique was used on agar plates of MRS (de Man Rogosa Sharpe Code: CM0361) medium (Oxoid); incubation was performed at 37 °C for 7 days. Liquid cultures were cultivated in microtube containing MRS CM0359 broth medium (Oxoid) and incubated on rotary shaker.

2.3. Physiological characteristics [8]

The physiological by determination of Gram was done by Gram Stain Method : Fix air-dried films of food sample in moderate heat. Stain films 1 min with crystal violet-ammonium oxalate solution. Wash briefly in tap water and drain. Apply Gram's iodine for 1 min. Wash in tap water and drain. Decolorize with 95% ethanol until blue color is no longer released (about 30 s). Alternatively, flood slides with ethanol, pour off immediately, and reflood with ethanol for 10 g. Wash briefly with water, drain, and apply Hucker's counterstain (safranin solution) for 10-30 g. Wash briefly with water, drain, blot or air-dry, and examine.

2.4. Biochemical characteristics of LAB [9]

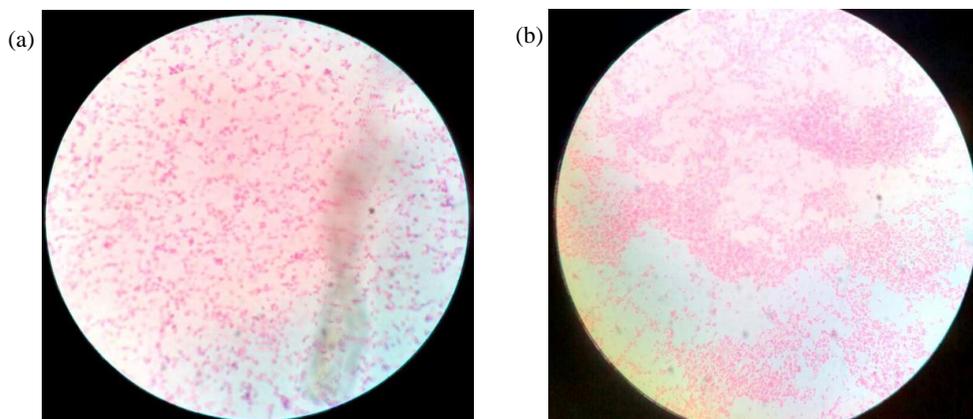
Biochemical characteristics of LAB were done by VITECH tools and then characterized using Bergey's Manual method.

2.5. Statistical Analysis

Those data were analyzed using descriptive method.

3. Results and Discussion

3.1. Physiological characteristics of strains



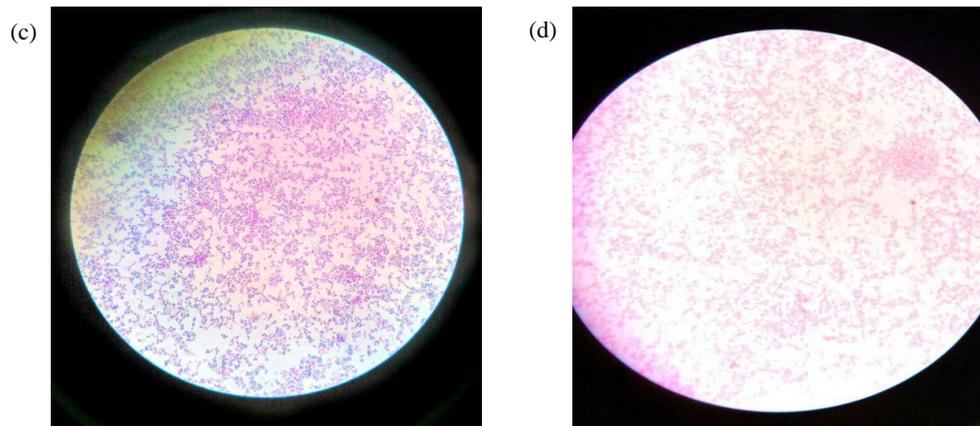


Figure 1. Gram staining of 4 strain isolated from shrimp paste, (a) LABS1; (b) LABS2 and (d)LABS4; (c) LABS3

Based on Figure 1, can seen that 3 of 4 cells strain (LABS1, LABS2 and LABS4) are Gram-positive, slender - rod - shaped, stricly aerobic, and red-pigmented, while the other one (LABS3) is dominated Gram-negative. Gram-positive cells found in shrimp paste samples with 60 days fermentation, while in shrimp paste samples with 30 days fermentation tends to Gram-negative with purple-pigment dominated. Those result indicated that different time of fermentation treated in the processing of shrimp paste influenced the presence of *lactic acid bacteria*. 30 days fermentation of shrimp paste allegedly did not optimum enough for microbes to produce secondary metabolites as well as fermentation time for 90 days, where secondary metabolite production tends to decrease. This decline is expected due to the availability of nutrients medium or shrimp paste samples are increasingly discharged by the 90th day so that no single isolates were found in shrimp paste samples with fermentation length for 90 days, and of course it would affected the glutamate levels of shrimp paste as a source of umami taste. The result are comparable to those obtained by previous study [7]. From 9 samples isolated, there were 4 presented of bacterial colonies and be expected as *Lactobacilli*. In fermented product, *Lactobacilli* had complex nutrient and requirement as indicator of many assays, such as the amount of amino acids in food products [10,11,12] as seen in Table 1.

Table 1. Microorganism used for assay of amino acids

Amino Acid	Microorganism
Arginine	<i>Streptococcus faecalis</i>
Aspartic acid	<i>Leuconostoc mesenteroides</i>
Cysteine	<i>Leuconostoc mesenteroides</i>
Glutamic acid	<i>Lactobacillus plantarum</i>

Glycine	<i>Leuconostoc mesenteroides</i>
Histidine	<i>Leuconostoc mesenteroides</i>
Isoleucine	<i>Lactobacillus plantarum</i>
Leucine	<i>Lactobacillus plantarum</i>
Lysine	<i>Leuconostoc mesenteroides</i>
Methionine	<i>Leuconostoc mesenteroides</i>
Phenylalanine	<i>Leuconostoc mesenteroides</i>
Proline	<i>Leuconostoc mesenteroides</i>
Serine	<i>Leuconostoc mesenteroides</i>
Threonine	<i>Streptococcus faecalis</i>
Tryptophan	<i>Lactobacillus plantarum</i>
Tyrosine	<i>Leuconostoc mesenteroides</i>
Valine	<i>Lactobacillus plantarum</i>

Banwart (1989)

It can be seen in Fig. 1 that the growth cells of strain be expected gave impact in cell change, whereas although *Lactobacillus* sp considered to be Gram-positive, as the culture ages, the cell may become Gram-negative [10].

3.2. Biochemical characteristics of LAB

Table 2. Characteristics of strain isolated from shrimp paste (*Shrimp paste samples, A3: 30 days fermentation for 1st repeated; B1: 60 days fermentation for 1st repeated; B3: 60 days fermentation for 3rd repeated); +: positive; -: negative

Characteristic	Isolated strains			
	LABS1	LABS2	LABS3	LABS4
Sample origin	*B1	*B1	*A3	*B3
Cell size (µm)	±0.1-0.3 x 0.8-2.5	±0.1-0.3 x 0.8- 2.5	±0.1-0.3 x 0.8-2.5	±0.1-0.3 x 0.8-2.5
Colony color	white	white	white	white
pH range for growth	5-9	5-9	5-9	5-9
Optimum pH for growth	7.0	7.0	7.0	7.0
NaCl range for growth (%)	10-15	10-20	10-20	5-15
Temperature range for growth (°C)	25-38	25-40	34-37	25-40
Optimum temperature for growth (°C)	37	37	37	37

Acid produce from :

D-fructose	+	+	-	+
D-galactose	+	+	-	+
D-glucose	+	+	-	+
Glycerol	+	+	+	+
D-mannitol	+	+	-	+
D-mannose	+	+	-	+
D-ribose	+	+	-	+
Sorbitol	+	+	-	+
Sucrose	+	+	-	+
Inulin	-	-	-	-
Lactose	-	-	-	-
Catalase	+	+	+	+
Oxidase	+	+	+	+
Urease activity	+	+	+	+
Nitrate reduction	+	+	+	+
Hydrolyses arginine and gelatin	+	+	+	+
Starch	-	-	+	-
Xanthine	-	-	-	-

Three strains tested positive. One strain tested negative

The G + C content of the type strain is 46 mol%

This study isolated 4 strains of *Lactobacilli*, Gram-positive rod vary from long to short coccobacilli, strictly aerobic, approximately 0.1 - 1.2 μm in diameter. Flagella were not observed. Colonies were clear, circular and white in colour. They grew in MRS medium CM 0359 and CM 0361 with 5-20% NaCl, at pH 5-9 and at 25-40 $^{\circ}\text{C}$, but no growth was observed at 10 or 50 $^{\circ}\text{C}$. The phenotypic and biochemical characteristics of the 4 strains are detailed in the species description below in Tables 2. Representative strain LABS1, LABS2 and LABS4 contained diaminopimelic acid in the cell-wall peptidoglycan. Positive in tests for catalase, oxidase, and urease activity and nitrate reduction. Hydrolyses arginine and gelatin, but not starch and xanthine. Produces acid from D-fructose, D-galactose, D-glucose, glycerol, D-mannitol, D-mannose, D-ribose, sorbitol and sucrose, but not from inulin and lactose. Phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids are predominant in the polar lipid profile. The DNA G+C content of the type strain is 41.6 mol%.

And as shown in Table 1 and the biochemical characteristics, 4 strains isolated from shrimp paste (*terasi*) samples represent a species within the genus *Lactobacillus*, for which we propose the name *Lactobacillus plantarum*.

4. Conclusion

This study conclude that strain *Lactobacillus plantarum* was dominant isolated from fermented shrimp paste (*terasi*) produced in Central Java . The secondary metabolites of *L.plantarum* was glutamic amino acid, which play roled as umami taste in shrimp paste.

5. Acknowledgement

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6. References

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