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Bacterial Pollution of a Traditional Terasi, Shrimp Paste Rebon (*Mysis relicta*)

Affan Gaffar
Department of Biology Education
Universitas Islam Negeri
Mataram, Indonesia
affan.gaffar@uinmataram.ac.id

Sri Sofiati Umami
Department of Biology Education
Universitas Islam Negeri
Mataram, Indonesia
Sofiati.umami@uinmataram.ac.id

Dadan Supardan
Department of Biology Education
Universitas Islam Negeri
Mataram, Indonesia
dadan.supardan@uinmataram.ac.id

Abstract*- Terasi is a fermented shrimp or fish or a mixture of both with salt. It is commonly used as cooking ingredient to make food more delicious. However, people are less informed on bacterial contamination on processed of Terasi that made by traditional household. This study was conducted to examine physical, biochemical and microbiology characteristic contained in Rebon shrimp paste and identify bacteria in the process of producing it. Three samples of Terasi were collected from different Districts in East Lombok. Six bacteria were screen and isolated by culture on Nutrient Agar Plate and grouped based on phenotypic, physiologic and biochemical characteristics. This study revealed that consisted bacteria of Terasi fermentation were identified as *Bacillus brevis, Bacillus polymyxa, Bacillus megaterium,* and *Staphylococcus aureus.

Keywords: Rebon shrimp paste, bacterial pollution, traditional food

I. INTRODUCTION

Terasi is a traditional product of fermented shrimps or fish with various concentrations of salt or other additives. Terasi has a strong characteristic odor and is usually used as a condiment to make chili sauce or found in various traditional Indonesian recipes, its main function is to give tasty and umami taste in food [1]. Terasi is widely consumed by people in Southeast Asia countries [2], but it has a different local name, *Belacan* in Malaysia and Brunei[3], *Bagoong* in Filipina, *Shajiang* in China, Ngapi in Myanmar, *Ka-pi* in Thailand and Cambodia[4], and *Mamtom* in Vietnam [1] [5].

Terasi or Shrimp paste is made of small fish or Rebon that has been processed through curing or fermentation, grinding or pounding, and drying, which lasts for 20 days. It is generally formed solid paste and has a reddish-brown color (Rebon) and black color (fish)[6]. In Indonesia, shrimp paste has been produced by homemade manufacture and has several level of quality of grade 1 to 3 depending on the ratio of shrimp and additional ingredients to the producing process[7].

Fermented Shrimp Paste, generally, has a moisture component of up to 70% and a total nitrogen content of almost 2%[8]. Shrimp paste also generates essential amino acids[3]. It also has considerably content of fat, protein and glutamic acid[9]. Lactid acid bacteria also obtained in Terasi, and is able to produce antibacterial activity of *lactic acid bacteriocin* [7]. In addition, food products such as shrimp paste are prone to bacterial contamination when manufactured traditionally. Previously study have reported samples of shrimp paste sold in some region in Surabaya containing Most Probable Number (MPN) coliform index exceeded the maximum limit of microbial contamination in food[10].

However, there is a few report regarding biochemical and microbiological properties of Terasi Rebon (shrimp paste) which could be contaminating to the product. Therefore, this present research was carried out for considering biochemical and microbiological properties of Terasi traditionally made by the local people in East Lombok. This result of this paper could give valuable information on bacterial contamination on processed food (Terasi) that is harmful to human.

II. METHOD

A. Isolation of Bacteria

Three samples of Terasi were collected from household manufacture in three different regions in East Lombok, Indonesia. Each of these (1g) was separately homogenized with steril mortar and pastle. Samples were macerated in 9 mL of NaCl (0.9%) then mixed by gently vortex. 1 mL of bacterial suspension were serially diluted in 9 ml of NaCl (0.9%) up to 10^{-5}. Aliquots of 0.1 mL suspension from each dilution were spread in duplicate on Nutrient Agar (NA) Medium and incubated at 37^0C for 24-72 hours. Colonies with different morphological character were streaked and purified on new fresh NA plates. Each purified colony was then stored on slants NA medium at 4^oC for futher analysis.

B. Morphological Characterization

The morphological profile was conducted by determining the configuration, margin, elevation, colour, and consistency of colonies according to Bergey's Manual of Systematic Bacteriology [11].

C. Gram Type Determination

The purified colonies were stained by Gram Stain which was conducted on 48 hour old cultures. A thin smear of pure isolates colony was stuck on a clean slide, dried in air and fixed by passing through flame of a Bunsen. The smear then was covered with few drops of crystal violet, let stand for one minute. The slide was washed with water, then covered with Gram iodine and kept for one minute. The slide was rinsed with water. Then decolourized with alcohol, was achieved by shaking the slide gently for twenty seconds till the violet colour came off the slide and then washed with water right away. Afterwards stained with safranin for twenty seconds. Washed with water, blot dried and then observed under the microscope.

D. Biochemical Characterization

Individual colonies were sub cultured on NB medium for biochemical screening.

III. RESULTS AND DISCUSSION

A. Isolation of Bacteria from Terasi

The isolation of Bacteria used the medium Nutrient Agar (NA) because it contains a sufficient source of nitrogen, 0.3% beef extract, and 0.5% peptone, but does not provide carbohydrate sources. This medium is suitable for bacterial growth, but not for mold and yeast. The colonies obtained from isolation were mostly round; only one isolate had an irregular shape. Likewise, the edge of the colony is primarily flat, one colony has an uneven edge shape, but the elevation or surface shape of the colony in all isolates is flat.

The colors of the colonies obtained in this study were different, most of which were creamy white, while others were clear, creamy white and golden yellow. Most bacteria have a whitish, gray, yellowish, or almost transparent color, but some species have a firmer color. The presence of color in bacteria is due to several external factors such as temperature, pH, and free oxygen. This study didn't measure those parameters.

Individual colonies were subcultured on NB medium for biochemical screening. In total, 6 different type colony were found. Biochemical tests, suspected isolates TA1, TA2, TB1, TB2, and TC1 are a group of Bacillus spp. The isolate of TC2 was suspected to be a group of *Cocci* spp. All isolates showed its ability to hydrolyze starch, which marked by the formation of clear zones around the bacterial growth area after being given a few drops of iodine Lugol solution. This clear zone shows that the bacterial isolate can produce the enzyme α-amylase, which can hydrolyze starch into simpler saccharides such as maltose and glucose [18].

Table 1. Characterization of bacterial isolates cultured from Terasi (Shrimp Paste Rebon)

Isolate	Texture	Colour	Shape	Elevation	Gram Staining	TSI	Simon Citrate	Urea	Motility	Glucose	Sucrose	Lactose	Maltose	Manitol	Indol	Metyl Red	VP	Pati	65% NaCl	Catalase	Spore	Identification
TA 1	Rough	Creamy White	Irregular	Flat	+	B/B	-	+/-	+	+	+	-	-	-	-	-	-	+	+	+	+	*Bacillus brevis*
TA 2	Smooth	Creamy White	Circular	Flat	+	A/B	+	-	-	+	+	-	-	+	-	+	-	+	+	+	+	*Bacillus megateriu m*
TB 1	Smooth	CreamyWhite	Circular	Flat	+	A/B	-	-	+	+	+	-	-	+	-	+	+	+	+	+	+	*Bacillus polymyxa*
TB 2	Smooth	CreamyWhite	Circular	Flat	+	A/B	+	-	+	+	+	-	+	+	-	+	-	+	+	+	+	*Bacillus megateriu m*
TC 1	Smooth	Creamy White	Circular	Flat	+	A/B	+	-	+	-	+	-	-	+	-	+	-	+	+	+	+	*Bacillus megateriu m*
TC 2	Smooth	Yellow	Circular	Flat	+	A/B	-	-/+	-	-	-	-	-	-	-	-	-	-	-	+	+	*Stapylococ cus aureus*

*NS; +:positive, -:negative results

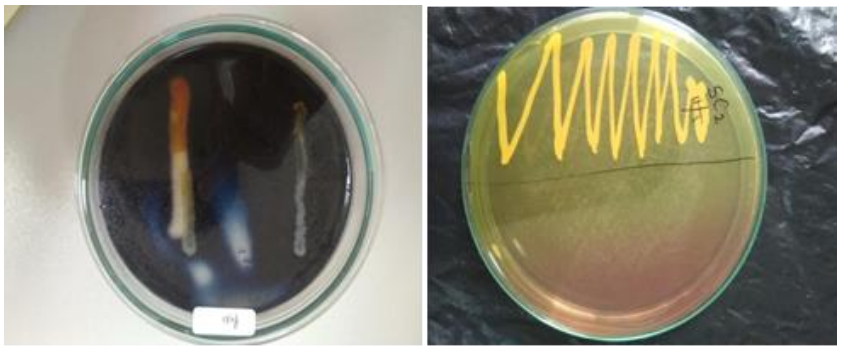
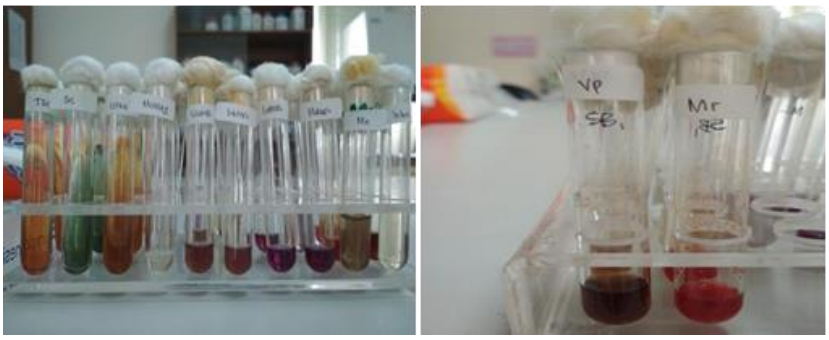


Figure 1. Growth response of bacterial isolate in presence of different media; Left: Starch hydrolysis; Right: Manitol Salt Agar (MSA)



igure 2. Growth response of bacterial isolate toward biochemical test : Triple Sugar Iron (TSI), Simon Citrat (SC), Urea, Motility, Glucose, Sucrose, Lactose, Maltose, Manitol, Indol, VP (Voges Proskeur), Metyle red.

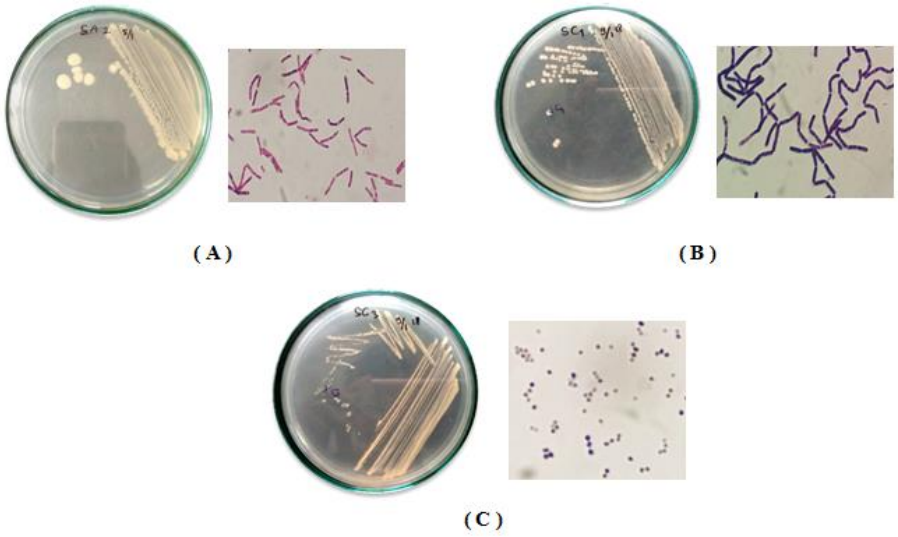


Figure 3. Morphological Characterization and gram stain for three bacterial isolates (A) TA2 (B) TC1 (C) TC2.

B. Identification of Bacteria

All of 6 isolates were examined for morphology, gram stain, biochemical and spore formation (see table 1). It was assumed that isolate TA1 was a *Bacillus brevis* bacterium. This bacterium is a Gram-positive, aerobic, and generate spore. *Bacillus brevis* is commonly found in soil, air, water, and rotting material. This bacterium is rarely associated with infectious diseases. *Bacillus brevis* is one of the bacteria that are able to produce antibiotic of gramicidin [19]. The presence of these antibiotics *Bacillus brevis* is able to inhibit transcription during the growth of gram-negative bacteria. In addition Bacillus brevis bacteria also produce Silver Nanoparticles (AgNPs) which used as an antimicrobial agent or that has the potential to fight pathogenic bacteria such as *Salmonella typhi* and *Staphylococcus aureus* [20].

Morphological and biochemical test results on isolates TA2, TB2, TC1 were suspected to be *Bacillus megaterium*. These bacteria are gram-positive include spore-forming *aerobic bacteria* found in very diverse habitats from the soil, seawater, sediments, rice fields, honey, fish, and dry food. *Bacillus megaterium* is

often used in laboratories as an industrial organism capable of producing various proteins and bioremediation sources. Proteins produced by these bacteria, for example, many synthetic penicillins are revealed to be penicillin amidases in bacteria; harvested glucose dehydrogenase is used in glucose blood tests; ß-Amylase which is often used in the bread and various food industries; and neutral proteases used by the leather industry [21]. The presence of several proteins produced by the bacterium *Bacillus megaterium* can be beneficial to the quality of shrimp paste.

While the results of morphological and biochemical tests on TB1 isolates were suspected to be *Bacillus polymyxa*. This bacterium is gram-positive, not pathogenic. *Bacillus polymyxa* is able to produce antibiotics in the form of *polymyxin* substances so that these bacteria are said to have the potential to prevent gram-negative bacteria[22]. The presence of *polymyxin* substances produced by these bacteria can inhibit the growth of gram-negative bacteria in shrimp paste. In addition, *B. polymyxa* can be used as a starter culture in inhibiting the accumulation of histamine during the fermentation process of shrimp paste products [23].

Morphological and biochemical test results on TC2 isolates, suspected to be *Staphylococci* bacteria group. This group consisting of *Staphylococcus*, *Micrococcus*, and *Aerococcus*. Microbiological Properties of *Staphylococcus* sp. and *Micrococcus* sp. almost similar, but based on the MSA test, the bacteria are *Staphylococcus aureus*. This bacterium is a normal flora found in parts of the human body such as hands, nose, mouth, and skin. The presence of *Staphylococcus aureus* in shrimp paste is thought to be due to contamination during the processing so that it will pose a risk of continuous food poisoning to human [24]. *Staphylococcus aureus* is still possible to grow in some products with a rather high salt content of less than 10%. However, the pathogenicity of the detected of *Salmonella aureus* in Samples of Terasi should to be examined.

IV. CONCLUSION

The predominant Bacteria consisted in fermented shrimp paste Rebon (Terasi) were *Bacillus* and Stapylococcus. According to morpho-physiological properties, six isolates were identified as *Bacillus brevis*, *Bacillus megaterium*, *Bacillus polymyxa*, and *Stapylococcus aureus*. Patogenic bacteria detected in Terasi was *Stapylococcus aureus*. Further research should be determining the pathogenicity of *Streptococcus* bacteria in Terasi samples so that the quality of traditional shrimp paste production can be improved.

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