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The Use of *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11 to Suppress the Population of *Salmonella – Shigella* and *Enterobacteriaceae* in Jambal Roti (Fermented Fish)

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ABSTRACT
Jambal roti is a fermented product from Manyung fish, which is quite famous in Java. The term 'jambal roti' refers to the salting and drying of fish. Manyung fish are easily damaged so that they need to be preserved by salting. The use of 30% salt in traditional production makes the product too salty. However, the decreasing amount of salt will lead to the growth of pathogenic bacteria such as *Salmonella - Shigella* and *Enterobacteriaceae*. This study examines the potential of *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11 in the making of jambal roti, with different variations of salt concentration (20%, 25% and 30%), to reduce the saltiness of the product and to eliminate the pathogenic bacteria. The results showed the use of *Pediococcus halophillus* FNCC-0032 (1.48 log CFU/gram to zero) with 25% and 30% salts compared to controls (without the application of *Pediococcus* sp)) and *Pediococcus acidilactici* F-11 could reduce the number of *Salmonella Shigella* bacteria. Compared to the control treatment, use of *Pediococcus* sp with 25% and 30% salinity (3.48 log CFU/gram to 2.0 log CFU/gram) could reduce the number of *Enterobacteriaceae*the amount of *Salmonella - Shigella* and *Enterobacteriaceae* tended to be stable (on 2.0 log CFU/gram) with the treatment of *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11 application at 20% salinity.

Keywords: *Pediococcus halophillus, Pediococcus acidilactic, Salmonella – Shigella.*

1. INTRODUCTION

Fish has been commonly used as a source of animal protein. Fish protein is easy to digest and contains amino acids with a similar pattern to those of in human body. There are several advantages of consuming fish meat as a protein source. Fish meat is a high protein product (20%), containing slightly woven binders so easily digested. Although the fatty acid is high (0.1 - 2.2%), 25% of this amount is an acid-unsaturated fatty acid that humans need. In addition, the cholesterol levels of fish meat are very low. Fish meat also contains a number of minerals (K, Cl, P, S, Mg, Ca, Fe, Zn, F, Ar,

Cu and Y) and vitamins A and D necessary for the human body. More importantly, fish meat can be consumed by all levels of society [6].

However, the fish body is high in water content (80%); the pH is close to neutral so it becomes a medium for mold, spoilage bacteria, and other microorganisms to grow. In addition, fish meat is susceptible to autolysis so it becomes a growth medium for microorganisms and mold.

Salted fish is a food made from fish meat that is preserved by adding a lot of salt. In this method of preserving, fish flesh which usually decays in a short time can be kept at room temperature for months. These fish are stored in a container then sprinkled in a concentrated salt solution. Due to the difference in concentration and osmotic pressure, salt crystals will pull the cell fluid in the fish meat out of the body. Meanwhile, the salt particles seep into the fish meat [5]. This process continues until there is a balance of salt concentration both inside and outside of the meat. Jambal roti is a salt fermented product made from sea catfish. In terms of its characteristics, jambal roti gives off a fragrant aroma from the degradation of protein and fat which produces methyl ketone, butylaldehyde, amino acids, and other compounds. In addition, the high content of nitrogen amino acids affects the taste of jambal roti. Another peculiarity is the soft and compact texture as a result of the work of proteolytic enzymes produced by microorganisms [1]

Pediococcus sp is a group of bacteriocin-producing homofermentative lactic acid bacteria (pediosin). The bacteria were isolated from fermented products. These bacteria have the ability to preserve food because they can produce lactic acid which can reduce the pH media and produce bacteriocin (pediosin). The presence of these bacteria could suppress the growth of other bacteria. *P. acidilactici F-11* bacteria have been used in the production of salted fish (inasua); they are able to suppress the growth of coliform bacteria and increase the content of lactic acid bacteria (Nendisa and Rahayu 2001).

Jambal Roti is a famous fermented fish product in Java, especially on the north coast of the island. Jambal roti is made with traditional methods and is prone to pathogenic bacteria as well as physical and chemical contaminants. This condition encourages efforts to suppress the growth of pathogenic bacteria (*Enterobacteriaceae* and *Salmonella-Shigella*) during the salting process. The salting process can suppress the growth of pathogenic bacteria; however, it still allows the growth of high salt resistant pathogenic bacteria.

The purpose of this study was to determine the effect of *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11 in the making of jambal roti with different salt variations (20%, 25%, and 30%) on the growth of *Salmonella-Shigella* and *Enterobacteriaceae bacteria*.

2. MATERIAL AND METHOD

2.1 Experiment design

The experiment was conducted with a completely randomized design (CRD) with 2 factors: variations in salt concentrations (20%, 25%, and 30%) and variations in *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11. Nine experimental groups were set to contain 600 grams of Manyung fish.

2.2 Lactic Acid Bacteria treatment

Pediococcus halophillus FNCC-0032 and *Pediococcus acidilactici* F-11 were bought from FNCC (Food Nutrition and Culture Collection), Gadjah Mada University. The Lactic Acid Bacteria had been inoculated in deMann Rogosa and Sharpe for 24 hours.

2.3 Manyung fish preparation

The research was conducted in May 2019. Manyung fish (*Arius thalassinus*) was bought from PT Holy Mina Jaya Rembang, Central Java, and stored in freezer box. Nine experiment groups were set with 600 grams of fish. Different concentrations of salt (20%, 25%, and 30% of weight of fish) were added during three days. Salt was bought from a local market. The fish was washed with water and had been inoculated in LAB culture (soaked) for 5 minutes. *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11 were inoculated 10^6CFU/ml in a culture medium for each experiment. They had been

dried using a sun-drying method for 3 day. Every day, 10-15 grams of each experiment was taken for analysis.

2.4 Microbial analysis

The *Enterobacteriaceae* analysis were performed with Violet Red Bile Agar (VRBA) (merck) media and whereas the *Salmonella-Shigella* analysis were performed with *Salmonella-Shigella* Agar (SSA) (merck) media. One of the samples is diluted on 9 ml steril NaCl (0.85%) until a 5-level dilution. 1 ml from 10^2, 10^3 and 10^4, was inoculated on a sterile petri dish and poured media (VRBA and SSA). The petri dish with sample had been incubated for 2 days; the numbers of colonies were then counted.

3. RESULTS AND DISCUSSION

1.1 Enterobacteriaceae

Enterobacteriaceae is a group of rod-shaped gram negative bacteria that are facultative anaerobes. These bacteria are categorized as the cause of most food-borne diseases. This large group of bacteria consists of non-pathogenic bacteria found in the body and the environment as well as pathogenic bacteria, such as *Salmonella*, *E. coli*, *Yersinia pestis*, *Klebsiella*, *Shigella*, *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter [7]*.

VRBA is a medium for the enumeration of coliform organisms in food and dairy products. The selectivity of the medium is due to the presence of bile salts and crystal violet. Lactose fermenters produce red/purple colonies often surrounded by a halo of the same colour [2].

Figures 1, 2, and 3 show the profile of *Enterobacteriaceae* in the process of making jambal roti with three different salt variations (20%, 25% and 30%). The number of *Enterobacteriaceae* in fresh fish was 3.4 log CFU/gram of samples, while the quality requirements of fresh fish according to SNI 2729: 2013 were less than 3 colonies of 1 gram sample, for *Escherichia coli*. The fish was freshly stored using ice cubes and then stored in the freezer [3].

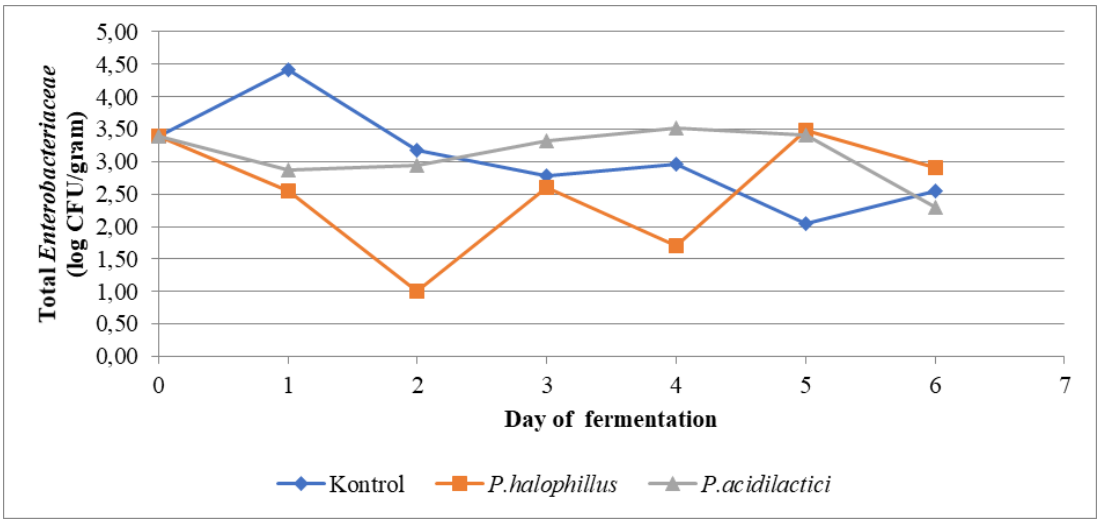

Figure 1. Number of *Enterobacteriaceae* bacteria on 20% salt content

During the fermentation process, the number of *Enterobacteriaceae* tended to decrease in all treatments. This decrease is influenced by the level of salt used; salt is able to suppress the growth of pathogenic bacteria such as *Enterobacteriaceae*.

Figures 1, 2, and 3 show the decreasing number (1 log cycle) of *Enterobacteriaceae*

(from 3.0 log CFU/gram to 2.0 log CFU/gram of samples) in the treatment with different variations in salinity and the supplementation of *Pediococcus halophillus* FNCC-0032, *Pediococcus acidilactici* F-11, and control. This showed that the salting can suppress the growth of *Enterobacteriaceae* in the making of jambal roti. The number of Enterobacteriaceae at the end of the treatment ranged around 10^2 CFU / gram (2.0 log CFU/gram). The value was below the SNI 2721: 2009 standard, the 10^5 CFU / gram amount of *Escherichia coli*.

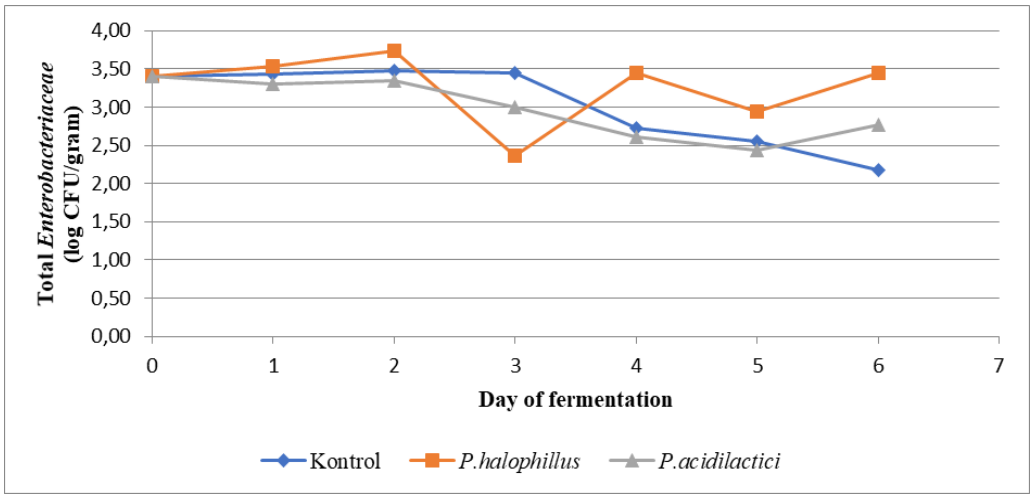

Figure 2. Number of *Enterobacteriaceae* bacteria on 25% salt content

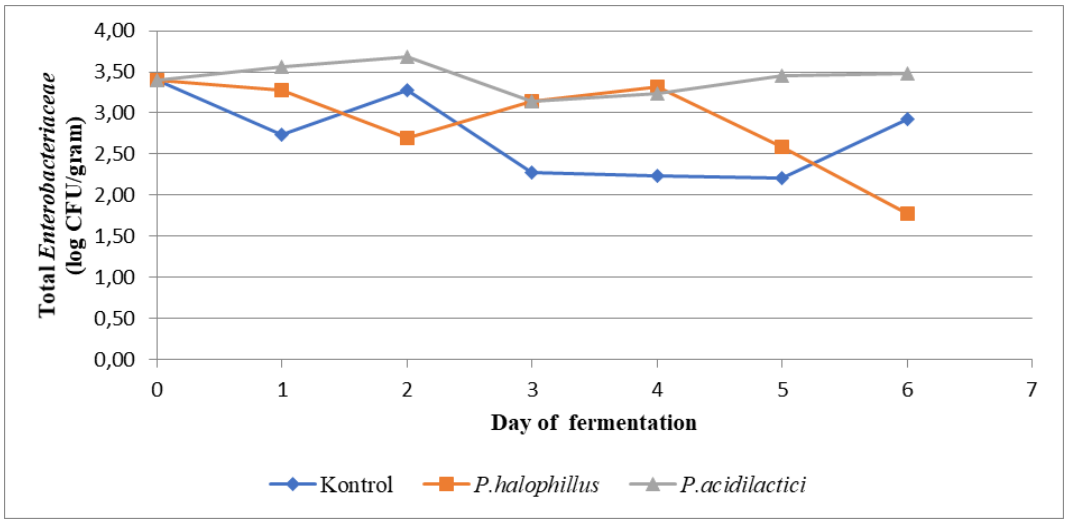

Figure 3. Number of *Enterobacteriaceae* bacteria on 30% salt content

Pediococcus sp is a group of lactic acid bacteria that can produce pediosin (bacteriocin) so that it can suppress the growth of other bacteria. One of which is pathogenic bacteria such as *Enterobacteriaceae*. *Pediococcus halophillus* (*Tetragenococcus halophilus*) is an important species in lactate fermentation used in the fermentation of products that contain high levels of salt (18 % NaCl). Pediococcus acidilactici F-11 have the ability to preserve food because it can produce lactic acid which can reduce the pH of the media and produce bacteriocin (pediosin), so that it can suppress the growth of other bacteria. Bacteria *P. acidilactici F-11* have been used in making salted fish (inasua) and are able to suppress the growth of coliform bacteria and increase the content of lactic acid bacteria [11]. Figures 1, 2,

and 3 show the ability of *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11 in suppressing the growth of *Enterobacteriaceae*.

The results showed that in the treatment using *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11, at the end of the treatment (day 6), the number of Enterobacteriaceae bacteria tended to be lower than the control treatment (without the supplementation of lactic acid bacteria). This shows that *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11 are able to suppress the growth of *Enterobacteriaceae* bacteria [9].

1.2 Salmonella – Shigella

Salmonella, Shigella, and *Yersinia* cause a well-characterized spectrum of disease in humans, from asymptomatic carriage to hemorrhagic colitis and fatal typhoidal fever. These pathogens are responsible for millions of cases of food-borne illness in the U.S. each year, causing substantial hospitalization costs and lost productivity [10]. In the developing world, illness caused by these pathogens is not only more prevalent, but it is also associated with a greater case-fatality rate [12]

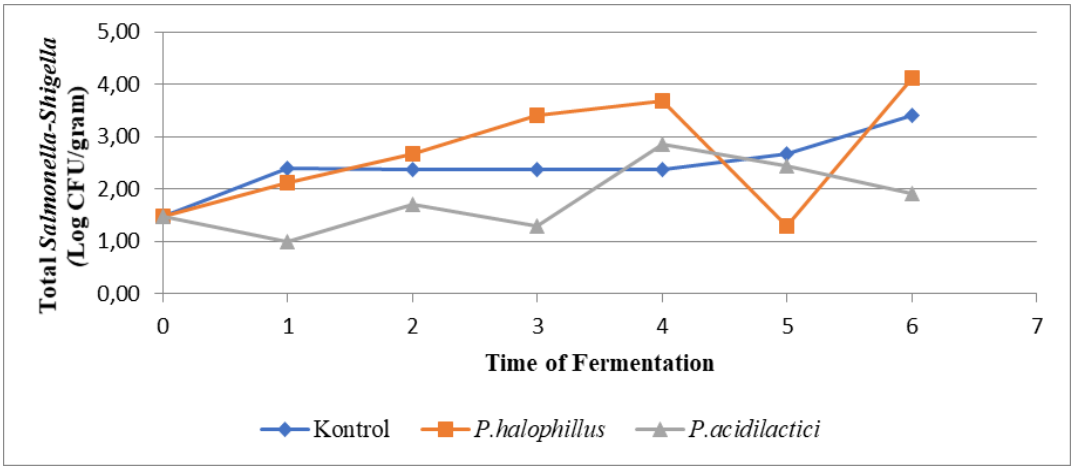

Figure 4. Number of *Salmonella-Shigella* on 20% salt content

Figures 4, 5, and 6 show the amount of *Salmonella* and *Shigella* in *Salmonella-Shigella* Agar media. The number of *Salmonella-Shigella* bacteria increases to 2 log cycles (from 1.48 log CFU/ gram to 3 log CFU/gram) in the treatment of salt variations and without the addition of *Pediococcus halophillus* FNCC-0032 and *Pediococcus acidilactici* F-11 [8]. This showed that salt could not suppress the growth of *Salmonella-Shigella* bacteria. SNI 2721: 2009 standard states that the amount of Salmonella in salted fish has a maximum of <3 MPN / gram, so that the number of *Salmonella-Shigella* is higher than the standard [4].

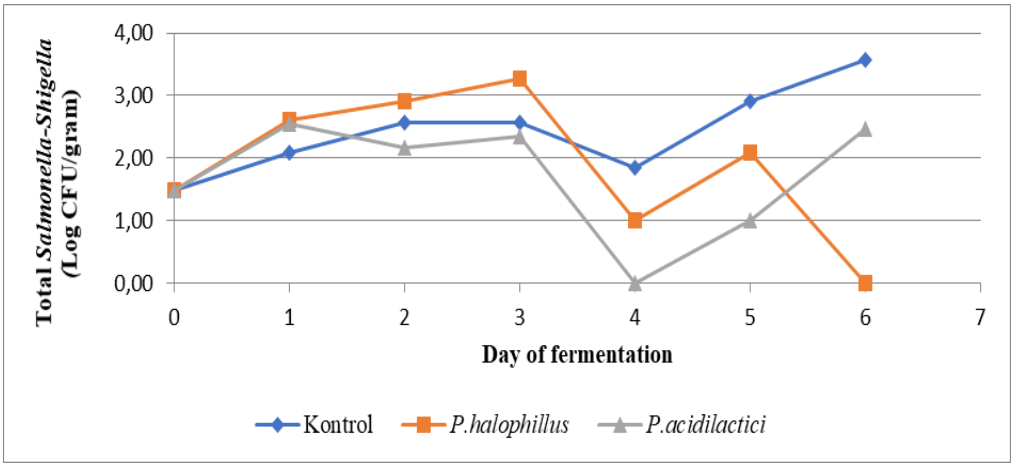

Figure 5. Number of *Salmonella-Shigella* on 25% salt content

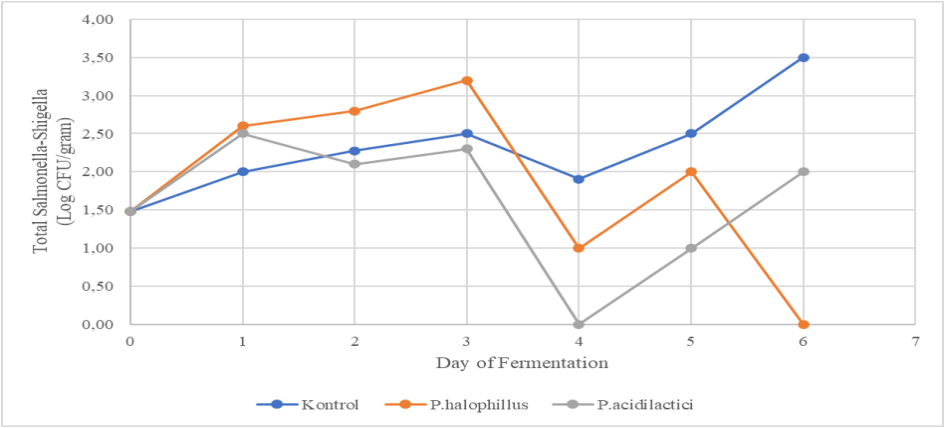

Figure 6. Number of *Salmonella-Shigella* on 30% salt content

Tables 5 and 6 shows that in the treatment of 25% and 30% salts and *Pediococcus halophilllus* supplementation, the number of *Salmonella-Shigella* at the end of treatment tends to be undetectable. Therefore, bacteriocins from *Pediococcus halophillus* FNCC-0032 can suppress the pathogen growth of Salmonella-Shigella. At 20% salinity, *Pediococcus halophillus* FNCC-0032 could not suppress *Salmonella-Shigella* bacteria.

In the treatment with the supplementation of *Pediococcus acidilactici* F11, the number of *Salmonella-Shigella* bacteria tends to increase. This is possible because the bacteriocins produced are less able to suppress the growth of *Salmonella-Shigella* bacteria.

4. CONCLUSION

Pediococcus halophillus FNCC-0032 and *Pediococcus acidilactici* F-11 are able to suppress the growth of pathogenic bacteria such as *Enterobacteriaceae* and *Salmonella-Shigella*, in all treatments.

ACKNOWLEDGMENT

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