Organized by:

RUMAH PUBLIKASI INDONESIA

CERTIFICATE

No. 3425/UN40.R4/DT/2020

This certificate is awarded to

N M Darmadi, D G S Edi and S A M P Suryani

as a **Presenter** of a paper entitled:

Bacteriocin antimicrobial isolation in fish soy sauce

in the 5th Annual Applied Science and Engineering Conference (AASEC) 2020 Universitas Pendidikan Indonesia "Green Technologies for Environmental Sustainability", 20-21 April 2020.

Prof. Dr. Didi Sukyadi, MA.
Vice Rector for Research, Partnership, and Business
Universitas Pendidikan Indonesia

Prof. Dr. Ade Gafar Abdullah, M.Si.
Conference Chair

the 5th aasec 2020 "Green Technologies for Environmental Sustainability"

Co-hosted by:

Green Conference

SURAT TUGAS
Nomor :333/UW-FP/PD-11/2020

Yang bertanda tangan dibawah ini :

Nama : Ir.Dewa Nyoman Sadguna,M.Agb

NIK : 230 500 084

Jabatan : Dekan Fakultas Pertanian Universitas Warmadewa

Dengan ini menugaskan,

No	Nama	NIP
1.	Dra.Sang Ayu Made Putri Suryani,M.Si	196704281992032001

Untuk melaksanakan tugas mengikuti seminar Internasional ***The 5th Annual Applied Science and Engineering Conference*** dengan tema ***"Green Technologies for Environmental Sustainability*** sebagai presenter, yang akan dilaksanakan secara daring pada :

Hari / Tanggal : Selasa , 21 April 2020

Waktu : 09.00-13.00 WIB

Tempat : Daring (Zoom Meeting)

Demikian surat ini kami sampaikan, untuk dilaksanakan sebagaimana mestinya.

Denpasar, 20 April 2020
Dekan,

Ir. Dewa Nyoman Sadguna, MAgb
NIK. 230 500 084

Tembusan : Arsip

Bacteriocin antimicrobial isolation in fish soy sauce

Bacteriocin antimicrobial isolation in fish soy sauce

N M Darmadi*, D G S Edi and S A M P Suryani

Study Program, Management of Aquatic Resources, Warmadewa University, Denpasar, Indonesia

*nimadedarmadi210466@gmail.com

Abstract. Fish sauce can be made from fermented fish. In fermentation plays a role Lactic Acid Bacteria (LAB). The role of lactic acid bacteria has a preservative effect because it can produce compounds that can inhibit the growth of various microbes. Besides lactic acid bacteria produce bacteriocin which also function as antimicrobials. The purpose of this study was to be able to isolate bacteriocin from fish sauce with SDS-PAGE electrophoresis. The study was conducted at the Laboratory of Agricultural Biotechnology Udayana University, Denpasar with a Descriptive Method. The research was carried out in sequence, namely, isolating lactic acid bacteria from fish sauce, Growing Lactic Acid Bacteria on Poor Nutrient Media so that its growth is depressed, because with life stressed Lactic Acid Bacteria can remove Bacteriocin as an Antimicrobial agent. With SDS-Page you will see separate proteins according to their weight, which are specific bands. The protein band which ranges from 17.0 kDa - 17.5 kDa is bacteriocin

1. Introduction

The portion of fish that could be consumed is merely about 40%, in which the other 60% is considered as waste. The waste should be handled through fermentation to increase its economic value. Fish waste contains many nutrients that can be utilized as liquidorganic fertilizer [1].

In addition, fish waste can also be made into fish sauce. Fish sauce has the characteristic of brown liquid containing dissolved protein and salt with the taste and smell of fish. In the fermentation technology, the role of Lactic Acid Bacteria (LAB) is very important, because the nature of LAB can suppress the pathogenic bacteria that cause diarrhea, as well as stimulate the immune system [2].

Lactic Acid Bacteria (LAB) are a group of bacteria that have the characteristics of a gram positive, negative catalase, do not form spores, and have colony with milky or slightly creamy color and a round shape [3]. The role of Lactic Acid Bacteria in fermentation process is to improve the taste of the product and also reduce the pH of the substrate so that it can suppress the life of pathogenic/decomposing microbes [4,5]. Fermentation also provides a preservation effect since fermentation produces organic acids such as Lactic Acid, Acetic Acid, and Butyric Acid. Lactic Acid Bacteria also produce hydrogen peroxide, diacetyl, carbon dioxide, reuterin and bacteriocin as anti-microbial substances. Nowadays, Bacteriocin is widely used as a bio preservative agent [6]. On the other hand, that Bacteriocin is considered to be quite expensive. Therefore, it is necessary to look for other sources that could produce Bacteriocin. In this research, it was tested whether the Lactic Acid Bacteria in fish sauce was able to produce Bacteriocin.

2. Methods

The study was conducted at the Laboratory of Agricultural Biotechnology and the Laboratory of Plant and Pest Diseases, Faculty of Agriculture, Udayana University, Denpasar. The study used a descriptive method with research steps: Making fish sauce, isolating Lactic Acid Bacteria, growing Lactic Acid Bacteria on nutrient-rich media and nutrient-poor media, isolating bacteriocin with the Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. The Proteins separation with SDS-PAGE includes three stages, namely: Protein Extraction, Gel Making and Protein separation with Electrophoresis to detect formed protein bands [7].

3. Results and discussion

3.1. Fish sauce making

Fish waste which was produced by fish traders in traditional markets and fish processors was considered to be abundant. If it was not handled properly, it would be able to pollute the environment. Fermentation technology was a solution for handling the fish waste. The fermentation process of the fish waste into fish sauce was done with the help of salt as the first filter for pathogenic microbes, as well as flavoring and extending the durability [7]. The fermentation process used also additional Bromelin enzyme which was derived from fresh pineapple extract. The enzyme was chosen because aside from being a protease, pineapple has distinctive fresh taste and aroma. With the aroma and taste, it was expected to affect the aroma and taste of the fish sauce product. The fermentation of fish waste with the help of the Bromelin enzyme lasts for four (4) days. Based on the chemical analysis, the fish sauce has a pH of 3.4, Acetic Acid 1.25%, Lactic Acid 1.26% and dissolved protein 5.3%. This happened because of the Lactic Acid Bacteria contained in fish sauce were included to both homofermentative and heterofermentative types, in which the bacteria could produce organic acids such as Acetic Acid and Lactic Acid that be able to reduce pH [8].

3.2. Lactic acid bacteria isolating

In isolating the Lactic Acid Bacteria of the fish sauce by using MRSA media—a special media for growing Lactic Acid Bacteria—the researchers used Pour method. Bacteria that have been grown were then identified through several tests such as: Gram Staining, Motility Test, Catalase Test, and Gas Production from glucose.

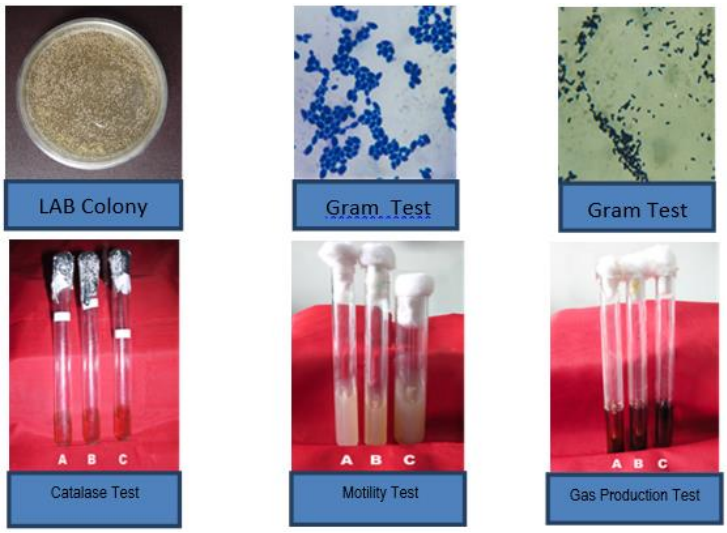

Figure 1. A series of lactic acid bacteria identification tests.

The results of the study found that the Lactic Acid Bacteria Genus contained in Fish Sauce were: Leuconostoc and Lactobacillus. A series of pictures of bacterial colonies and a series of lactic acid bacteria identification tests could be seen in the Figure 1.

3.3. Growing lactic acid bacteria isolates in different media (Nutrient-rich media and nutrient-poor media)

Bacteriocin is an extracellular metabolite in the form of a protein that is synthesized directly in Ribosome and has varied activity in a broad spectrum of antimicrobials [9]. Bacteria will release metabolites if they are depressed. In this study, to observe whether the Bacteriocin were produced or not, the researchers should grow the LAB in different media. The LAB isolates were first grown on the same medium, which were LB for 24 hours. After the LAB were growing well, then they were grown separately on different media.

The nutrient-rich media was using the composition of MRS broth (in 1 Liter): Bacto peptone 10 g, Meat extract 10 g, Yeast extract 5 g, K2HPO4 2 g, Ammonium citrate 2 g, Glucose 20 g, Sodium acetate 5 g, Mg SO_4 $7H_2O$ 0.58 g, MnSO4 $4H_2O$ 0.28 g. Whereas, the nutrient-poor media was using the same composition of MRS broth in which some components were not included, e.g. without meat and without glucose added.

The bacterial growth was observed every 6 hours and the absorbance was seen on a spectrophotometer with a wave length of 600 nm. If the bacteria have undergone a Stationary Phase (about 96 hours), it was estimated that the Bacteriocin has been produced. Therefore, the Bacteriocin isolation could be begun.

3.4. Bacteriocin followed by SDS-PAGE

After the bacteria underwent a stationary phase, each culture was taken 1 eppendorf and centrifuged at 5000 rpm for 15 minutes. Move the supernatant to another eppendorf. The supernatant was thought to contain secondary metabolites produced by bacteria that were grown. 600 µl of supernatant from the nutrient-rich media and nutrient-poor media was taken, each added 300 µl of buffer sample (0.01 M Tris, 2.5% SDS, 5% Mercaptoethanol and 25% sucrose) and then added dye of 300 µl Comassie blue (1 g Comassie Brilliant Blue, 200 ml of Methanol, 40 ml of acetic acid). Those suspensions were placed in eppendorf (1.5 ml) and were vortexed, then centrifuged at 5000 rpm for 2 minutes and incubated at 95° C for 5 minutes. The supernatant obtained was used to observe protein separation by using the SDS-PAGE method. The protein separation by polyacrylamide gel electrophoresis occured based on the differences in charge and molecular size. The polyacrylamide gels were formed by the polymerization of acrylamide and bis-acrylamide. This polymer formation reaction was initiated by a system that produced free radicals. The lower the concentration of acrylamide used the larger the pore size [10].

3.5. Separation of protein molecules with SDS-PAGE

Procedures used for the separation of protein molecules according to Coyne et al. [11]. Using Resolving gel with a concentration of 12% consisting of 4.57 ml distilled water, 2.73 ml Acrylamide mix, 1.5 M Tris pH 8.8 (2 ml), 10% SDS (0.1 ml), 10% ammonium persulfate (0.1 ml), 0.01 temed.

Stacking gel with a concentration of 4% containing 6.4 ml of distilled water, 0.9 ml of acrylamide mix as much as 2.5 ml of 0.5 M Tris pH 6.8, 10% SDS (0.1 ml), 10% ammonium persulfate (0.1 ml) and 0.01 ml temed. Electrophoresis was carried out at a voltage of 50 V and the voltage was increased by 70 V after the samples entered the resolving gel, and the electrophoresis was stopped when the dye had reached the bottom of the resolving gel. After the electrophoresis process was completed then the staining stage was carried out. In the staining stage, the gel from the electrophoresis tools were immersed in a coomassie Brillian Blue (CBB) solution for 1-2 hours. CBB binded to protein molecules when washed with methanol, so that the protein bands appeared clearly. For the first washing, the gel was washed with destaining solution containing 250 ml of methanol and 70 ml acetic acid which has been dissolved in distilled water to a volume of 1000 ml for 30 minutes. For the second washing, the gel was washed with the same solution, however should be carried out for 12 hours (overnight). And then, the

gel should be rinsed with aquadest and stored in a plastic bag. The results of electrophoresis could be seen from the position of the band of each sample which was compared with marker. Antimicrobial molecular weight produced by lactic acid bacteria ranges from 1700-100000 Dalton [12]. The results were shown in the pictures below.

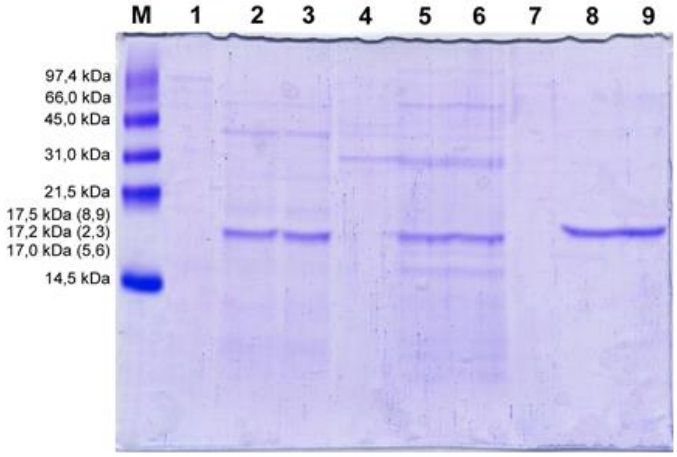

Figure 2. Lactic acid bacteria protein bands in fish sauce.

Based on the pictures, it could be explained that M was a Marker, columns 1, 4 and 7 were the LAB isolates grown in nutrient-rich media, whereas columns 2, 3, 5, 6, 8, 9 were LAB isolates grown in nutrient-poor media. Apparently, the LAB isolates grown in nutrient-poor media produced bacteriocin with molecullar weight of protein 17.2 kDa.

4. Conclusion

Based on the result of the study could be concluded that the Lactic Acid Bacteria found in fish sauce can produce bacteriocin with a protein molecular weight of 17.2 kDa.

Acknowledgments

Thank God for giving us the ability and opportunity to complete this paper. Special thanks to the Chairperson of the Bali Province KORPRI Welfare Foundation, Rector of Warmadewa University, and Dean of the Faculty of Agriculture. We really appreciate friends for their support, advice, and contribution in completing this research.

References

[1] Suartini K, Paulus H, Abraham and Jura M R 2018 *Jurnal Akademika Kim* **7** 2 70-74
[2] Rahmiati R and Mumpuni M 2017 *Elkawnie* **3** 2 141-150
[3] Putri Y W, Putra A E and Utama B I 2018 *Jurnal Kesehatan Andalas* **7** 20-25
[4] Dali F A 2013 *Jurnal Pengolahan Hasil Perikanan Indonesia* **16** 2
[5] Yuniati F A A and Rasmi D A C 2014 *Jurnal Biologi Tropis* **14** 2
[6] Romadhon R, Subagiyo S and Margino S 2012 *Indonesian Journal of Fisheries Science and Technology* **8** 1 59-64
[7] Utomo W T, Suarsana I N and Suartini I G A A 2017 *Jurnal Veteriner Juni* **18** 2 232-238
[8] Mergypta D, Budiharjo A and Kusdiyantini E 2014 *Jurnal Akademika Biologi* **3** 2 11-19
[9] Sunaryanto R and Tarwadi T 2015 *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan* **10** 1 11-18
[10] Widia S 2001 *Biokimia Eksperimen Laboratorium* (Jakarta: Widya Medika)
[11] Coyne V E, James M D, Reid S J and Rybicki E P 2003 *Molecular Biology Techniques Manual* (University of Cape Town)
[12] Bhunia A K, Johnson M C and Ray B 1987 *Journal of Industrial Microbiology* **2** 5 319-322