Review Article

A REVIEW: FERMENTATION MICROBIALS ISOLATED FROM MARINE

ABSTRACT

Marine fish is one of the foodstuffs that contain many compounds that are good for the human body. Marine fish contain omega-3 essential fatty acids (DHA) which are few other animal protein ingredients. This fish is rich in nutrients and minerals that can help prevent and maintain the body's immune system so that the body is fit. The benefits obtained include the maintenance of the cardiovascular system, efficient brain function, preventing damage to the thyroid gland and also improving eye health. Fermentation is one way to process fishery products by utilizing the decomposition process of compounds from complex protein ingredients. To ferment food, microorganisms are needed that play a role in the decomposition of organic components in the material. Microorganisms commonly used are lactic acid bacteria (LAB), acetic acid bacteria (BAA), bacilli or other bacteria, yeast, or filamentous fungi.

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Keywords: fermentation, fisheries, isolated, microbials

1. INTRODUCTION

As interest in marine aquaculture increased, the production of fish for the consumer market also resulted in remarkable gains in the ability to keep marine fish species in captivity (Purdom & Preston 1977). Despite these developments, the microbiological aspects of agricultural marine fish and, more importantly, the involvement of bacteria in fish diseases, have received little attention.

Detailed information on the number and types of bacteria in an unpolluted marine environment is essential if abnormal conditions such as: possibly caused by adverse water quality factors or the onset of favorable disease conditions, are to be recognized and corrected. Emphasis has been placed on the number (Oppenheimer 1955; Patrick 1978) or bacterial type (Baumann et al. 1971, 1972; Austin et al. 1979a) but there are no data on seasonal shifts in microbial populations that might be expected under marine conditions (Pfister & Burkholder 1965). ; MacLeod 1965, 1968; Baumann et al. 1971; Austin et al. 1979a).

Fermentation is the oldest food processing method in the world. Fermented foods have been around since 6000 BC. The first fermented product discovered in India was fermented milk which produces curd. Then

came fermented cheese, wine, bread, sausage, beer, sauerkraut, yogurt and so on. For each product, the microorganisms that play a role in fermentation are different. This depends on the diversity of microflora in each different area. In Indonesia, there are also various kinds of fermented foods, including brem, pickled vegetables and fruit, curd, tempeh, oncom, tape, tempoyak, and so on.

According to biochemists, fermentation is the process of forming ATP, an organic component that acts as an electron donor and acceptor. The popularity of fermented foods is a concern, so much research has been done. Its ability to enhance and enhance shelf life, nutritional value, health effects, and sensory properties make fermented foods interesting to study. Fermented milk products fermented by lactic acid bacteria Lactobacillus helveticus are thought to be able to lower blood pressure.

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To ferment food, microorganisms are needed that play a role in the decomposition of organic components in the material. Microorganisms commonly used are lactic acid bacteria (LAB), acetic acid bacteria (BAA), bacilli or other bacteria, yeast, or filamentous fungi. These microorganisms were first isolated and characterized by Pasteur, Lister, and other microbiologists.

Lactic acid bacteria are a group of gram-positive, non-spore-forming, aerotolerant bacteria (phylogenetically included in the phylum Firmicutes and the order Lactobacillales). These bacteria are widely used in food fermentation and play an important role in the fermentation of dairy products, cereals, meat and vegetable products. Apart from lactic acid bacteria, several species of Bacillus play a role in the fermentation of certain foodstuffs, for example Bacillus subtilis is used in fermenting soybeans to produce natto, and acetic acid bacteria are used in the manufacture of vinegar. In addition, Staphylococcus, Enterococcus, Brevibacterium, and Propionibacterium also have a supporting role in the fermentation of sausages and cheese. In fermenting cabbage or other green vegetables, the microorganism involved is Leuconostoc mesenteroides followed by Lactiplantibacillus species, and Levilactobacillus brevis which produces sauerkraut (from Europe and North America), kimchi (from Korea), suan-cai (from China) or sinki. (from China). from Nepal). Within the group of fungi, yeasts that produce alcohol, usually Saccharomyces, are used in the manufacture of bread, beer, wine, and other alcoholic fermentations. Other groups of fungi such as Penicillium, Aspergillus, and Rhizopus are able to ferment dairy products, meat, and soybeans as well as strains that produce proteinases, lipases and amylase.

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2. FISHERIES FERMENTATION

Fermentation is one way to process fishery products by utilizing the decomposition process of compounds from complex protein ingredients. Complex proteins are found in fish bodies that have been converted into simple compounds with the help of enzymes originating from the body of fish or microorganisms that have taken place in an orderly state.

Fermentation can be divided into 2, namely: fermentation that may occur as a decomposer or transformation which can produce products with different shapes and properties from the beginning or decomposition occurs, such as: Terasi, Fish Soy Sauce and Ikan Peda. Fermentation that can only produce compounds that can make the product last longer in its processed products, such as making salted fish peda.

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Fermentation of fish is a biological decomposition process because it changes protein complex compounds into simple compounds under controlled conditions. Then followed by amino acids that break down and become components that have a role in the formation of the taste of the product.

In the fish fermentation process, which is a biological process based on existing principles, it can be divided into 4 groups, namely: Fermentation with high levels of salt, such as: fish sauce, shrimp paste, shrimp and peda fish. Fermentation with organic acids, such as: making fish silage by adding propionic and formic acids. Fermentation with mineral acids, such as: making fish silage using strong acids. Fermentation with bacteria, such as: making tamarind and chao anchovies. Fermentation with a high salt content will make the protein present in fish taken a little by the salt, but salt is also added to stimulate the growth of lactic acid bacteria.

Types of fermentation that can be carried out on fish are: salt fermentation which usually uses dry salting for fish with low fat content or wet salting for fish with high fat content, salting has the function of: increasing the taste of fish; forming fish texture; control the growth of microorganisms by stimulating them, and can inhibit the growth of spoilage and pathogenic microorganisms. The second is lactic fermentation which occurs due to the activity of lactic acid bacteria, there are 2 groups: homofermentative lactic acid bacteria and heterofermentative lactic acid bacteria.

3. FERMENTATION BACTERIA AND ISOLATION METHODS

3.1.1. FLAVOBACTERIUM SP

Flavobacterium is a genus of Gram-negative bacteria (bacteria which when observed under a microscope are red or purple in color because these bacteria do not retain crystal violet dye during the gram staining process), rod-shaped, non-motile and motile, and this bacterium has 130 species. Flavobacterium is spread in nature, usually found in wet aquatic ecosystems, either fresh water or sea water or soil. Flavobacterium columnare is one of the bacteria that causes Columnaris disease from the Family Flavobacteriaceae (Bernardet and Bowman, 2006) and this bacterium is a species that often attacks freshwater fish (Durborow et al., 1998). These bacteria can live in all water areas, which can affect fish found in nature and aquariums (Austin *and* Austin, 1999).

Methods Isolation of *Flavobacterium columnare* can be carried out using fish for bacteriological samples in the form of a collection of skin and kidney samples as many as 100 samples collected from 50 near-dead fish from farms monitored by the Laboratory of Welfare and Disease Control of CEPTA/IBAMA characterized by rotten fins. and other skin lesions, such as grayish-white spots, especially on the head and erosions of the dorsal skin just in front of the tail and dorsal fin were selected for this study (Valenciennes, 1850). All fish were collected alive and examined for 24 h. . Collected fish were subjected to extensive macroscopic examination to record clinical health status. Mucus samples were obtained by skin scraping from the head, gills, caudal and dorsal fins and wet mounts were used to determine motility in the hanging-drop method and Gram stain was used to determine the Gram reaction. The material is examined with a phase contrast light microscope. Following this, skin and kidney samples were taken aseptically, with a direct swab of tissue exposed by a dorso-ventral cross section into the body cavity carefully to avoid cutting the intestine. These samples were streaked onto broth or modified agar from Anacker and Ordal (1955), a non-selective medium for the isolation of slide Gram negative bacteria and incubated at 25 °C for 48 h and the suspected colonies were further analyzed.

The medium used in this study was a modification of that used by Anacker and Ordal (1955) to induce the growth of other bacteria and limit the growth of Flavobacterium. This modification of Anacker and Ordal (1955) media was found to be very satisfactory for recovering columnar Flavobacterium. The modified media consisted of: peptone 3 gL–1, mushroom extract 5 mL.L–1, sodium acetate, 0.02 gL–1, NaCl 0.02 gL–1, fish peptone 100 mL.L–1 and agar 2 ,5 gL–1. pH was adjusted to 7.2 with NaOH. Infusion of fish peptone was

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prepared with muscle pieces and scales of Oreochromis niloticus (Linnaeus, 1757) (about 15 g each) in 1000 mL distilled water. Each part of the extracted muscles and scales was ground for twenty minutes and boiled for five minutes. The liquid was allowed to stand for thirty minutes and then filtered. The infusion was added to the broth and solid medium and autoclaved at 120 °C for 15 min.

After the strains were grown in liquid medium for 48 hours, their characteristics were evaluated along with their growth appearance in a liquid medium flask culture, the morphology of Gram staining bacteria was observed under a microscope (magnification, x 100) and typical stretching movements. bacteria using a hanging dropper and a light microscope (magnification, x 40). Ten-fold serial dilutions of each culture were made in phosphate-buffered saline, and 0.1 mL of each dilution was inoculated on five different Anacker and Ordal plates. All plates were incubated at 25 °C for 48 h. After incubation, viable counts are carried out simultaneously on plates. The identification of the isolated Flavobacterium strain was examined based on the characteristic yellow-green colonies, with rhizoid edges containing Gram-negative rods indicating gliding motility (Shamsudin and Plumb, 1996). After colony purification, the four isolates were identified using the criteria previously described by Bernardet and Grimont (1989).

In the study of morphology and cellular physiology were determined by Gram stain and bacterial cells were observed under a microscope (magnification, x 100). Motility was determined on a wet mounted under a phase contrast microscope (magnification, 40x). Other tests used were: colony appearance and consistency, pigment production after exposing the culture to KOH solution (20%). Glide motility was examined under a microscope (magnification, x 100) using a hanging drop preparation of 48 h liquid culture medium. The presence of flexirrubin-type pigments, Congo red absorption, catalase, DNAse, starch hydrolysis, and gelatin liquefaction were all as described by Reichenbach et al. (1974).

The presence of catalase was determined with hydrogen peroxide. The degradation of gelatin was tested with a liquid medium supplemented with 0.4% (wv–1) agar which was flooded with mercuric acid chloride. Gelatin degradation is indicated when local cleaning is observed in the media. The production of acid from glucose was determined using a liquid medium plus 0.4% dextrose. Starch hydrolysis was determined using a medium containing 0.2% (wv-1) soluble starch solution in which the colonies hydrolyzing starch produced clear areas. The casein hydrolysis was determined with agar medium supplemented with 10% dry milk. Plates were flooded with 1% HCl five days after inoculation, in which areas clearly showed proteolysis. Absorption of Congolese red was assessed by flooding the plate containing the colonies with a 1.0% Congolese red solution for several seconds, followed by rinsing with tap water.

Colonies possessing the flexirubin type of pigment showed an immediate color change from yellow or orange to red, purple or brown when flooded with 20% KOH and returned to their initial color when flooded by an acid solution (Bernardet et al., 2002). The ability to reduce nitrate was investigated in a 0.1% potassium nitrate broth tube (Bullock, 1972). Other biochemical tests were determined using the *Enterobacteriaceae*.

3.1.2 HOLOBACTERIUM SP

Halobacterium bacteria according to Wan Setiawan (2009) are halophilic bacteria or bacteria that are able to withstand high salt and can almost be found in waters that have a fairly high salt concentration. The characteristics are round wavy, white colonies, jagged edges and bacterial cells have a gram-negative bacillus (stem) shape, cannot ferment lactose and sucrose, and Halobacterium does not use citrate as a carbon source and is non-motile.

The tools used in isolating these bacteria are petri dishes, measuring cups with sizes of 50 and 100 ml, 500 ml beakers, 250 ml Erlenmeyer, volume pipettes with sizes 5 and 10 ml, test tubes, dropper pipettes,

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Bunsen burners, tubes. Durham, ose needle or bacteria growing device, magnetic stirrer, suction rubber, micro pipette and spray bottle. Then the incubator, autoclave, analytical balance, light microscope, gas stove or heater and oven and refrigerator.

The ingredients used are 3% hydrogen peroxide, sodium and cellulose agar, sodium chloride with 0.9%, 70% and 95% ethanol, glucose, lactose, sucrose, methyl red, , iodine solution, mannitol, maltose, Lugol's solution. safranin iodine and aquades.

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One way that can be used to isolate these bacteria is the Platting Method or the dilution method. This dilution method can be done by shaking or uniting the sample as much as 1 gram with 0.9% NaCl solution in a test tube until homogeneous. Then take 1 ml of the homogeneous solution, then add 9 ml of physiological NaCl solution and a dilution of 10^{-1}. If it has been obtained, then to obtain the results of a 10^{-2} of a dilution sample from 10^{-1} is taken and added with 9 ml of physiological solution in a test tube. Likewise for so on until a dilution of 10^{-9} obtained. Then transfer the results of the dilution using a pouring cup to culture which has been added with fungicide. Then incubate at 30^{o}C for about 1 to 2 days. When finished, observe the growing colonies based on shape, texture, size and color using a microscope. And to obtain these isolates carried out by the purification stage.

3.1.3 MICROCOCCUS SP

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Micrococcus is a variety of microscopic organisms in the family Micrococcaceae. Micrococcus occurs in a variety of conditions, including water, residue, and soil. Micrococci have Gram-positive spherical cells that are 0.5 to 3 micrometers apart and generally appear as quadruples. They are catalase positive, oxidase positive, indole negative and citrate negative. Micrococci have significant cell divisions, which may contain as much as 50% of the cell mass. The Micrococcus genome is rich in guanine and cytosine (GC), typically displaying a GC content of 65 to 75%. Micrococci often carry plasmids (changes from 1 to 100 MDa) that give life forms valuable characteristics.

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Some types of Micrococcus, such as M. luteus (yellow) and M. roseus (red) produce yellow or pink provinces when grown on mannitol salt agar. M. luteus isolate has been found to overproduce riboflavin when grown on naturally toxic toxins such as pyridine. The hybridization study showed that the species in the Micrococcus species were not closely related, only half of the kinship arrangement appeared. It proposes that some Micrococcus species may, based on examination of ribosomal RNA, be renamed in the long term to other microbial genera.

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For the confinement of microbes Micrococcus luteus and Staphylococcus epidermidis in this study, Aceh cattle udder swabs were obtained from UPT Experimental Animals, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh. The samples were then taken to the Microbiology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University for separation and identification of microorganisms.

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The equipment used in the isolation of micrococcus bacteria include: sterile swabs, tubes, hatchery, sanitizers, magnifying lenses, object glass, cover slips, droppers, test tubes, tube racks, Durham tubes, marking paper, spirit, autoclave and coolers. The material used in this study was udder swabs of 10 Aceh dairy cows. The media used for the separation of Gram positive microorganisms were Nutrient Broth (NB), Mannitol Salt Agar (MSA), Blood Agar (BA), Sodium Agar (NA) impurities, Gram staining arrangement media (precious stone violet, lugol, safranin, 96% liquor). , physiological NaCl), 3% H2O2 composition, soaking oil, and sugar medium (mannitol and glucose).

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The isolation method uses the Carter strategy. The samples taken were separated as indicated by the number of cow ears. The sample obtained was then put into a test tube containing NB. Then, at the time, it was limited to MSA-only media and BA media. The results of the assessment were carried out to identify microorganisms using Gram staining, followed by a catalase test, and a sugar test (mannitol and glucose).

The test ended with a cow udder swab using a q-tip to separate the microscopic organisms M. luteus and S. epidermidis and then put them into the supplement stock (NB) media. The confinement was carried out through the udder swab test which was immersed in NB media, then incubated at 37 °C for 24 hours anaerobically using a fire compartment. Furthermore, using ose, these groups of bacteria were immunized on MSA media using the quadrant scratch technique, then incubated for 24 hours at 37 °C. The different state microscopic organisms were then grown in dirty NA for use as stock.

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The gram staining process is accomplished by taking one to two drops of distilled water and dripping onto a slide, then, at that time, taking the place apart from each group of bacteria using an ossicle and then circulating them evenly. The bacterial smear was allowed to dry and fixed. Then, at that time, the microorganism smear was dripped with gem violet solution for 3-5 minutes and drained with running water. The smear is then dripped with Lugol's solution for 1 second, then at that time 96% liquid is added for 10 seconds until the color is not blurred and flows with running water. The last stage of the staining system is to add correlation dye, become specific safranin for 1 second and recirculate with running water. Then it was dripped with puddle oil, then seen the shape and shadow of the bacterial cells under a magnifying lens with 1000x amplification (Agustina et al., 2013). One culture circle was taken from NA impurities, then streaked on BA media by forming quadrant streaks.

3.1.4 CORYNEBACTERIUM SP

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Corynebacterium diphtheria is a bacterium that can be called a bad, harmful bacteria or better known as an antagonist, the shape of this bacterium is a bacillus or rod and has gram-positive properties, this bacterium has a size of 0.5-0.9 m this type of bacteria is immobile, Antagonistic bacteria can produce antibiotics. (Kambang Sariadji RH, 2013).

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The method usually used for isolation of Corynebacterium diphtheria is with cystine tellurite blood agar medium, using the blood agar method. this will cause other bacteria will not to grow and only Corynebacterium diphtheria bacteria, and has been recommended to be the most optimal method for culturing Corynebacterium diphtheria bacteria, if humans are infected with this bacteria it is likely to experience Diferi disease.

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In isolating Corynebacterium diphtheria the steps that must be taken are the first to instill bacteria in the media, namely so that this medium will later become a place for the growth of the bacteria, incubation for 24-48 hours at a temperature of 370 C usually bacteria that have grown have The characteristics of the colonies are round colonies, where the colonies have a smooth surface and have a dark grayish color, this black color is formed because cysteine tellurite turns into potassium tellurite.

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Next, staining is done on bacteria when staining with Neisser on rods that have metachromatic granules and will be colored purple bacteria at the ends, the existing colony shape is like the letter V and uniquely shaped like Chinese letters, then after staining is carried out catalase testing and is characterized by the presence of bubbles in the water, the bacteria have catalase enzymes and hydrogen peroxide (H2O2), then a coagulation test is carried out when there is no precipitate then it shows negative coagulation.

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Furthermore, toxigenicity testing was carried out with the Elek test with the container, namely Petri dish and added antitoxin 25 IU ADS in the middle of the Petri and the isolate was placed around the antitoxin at a distance of 1 cm then incubated for 24-48 hours at 370 C (Kambang Sariadji, 2017).

3.1.5 BACILLUS SP

The morphology and characteristics of this bacterium Bacillus sp is having a whitish color with a round body shape. In addition to its round body shape, the shape of this bacterial colony is round and white and, if you look at the characteristics of this bacterium, it belongs to the Bacillus group of bacteria. Corbin in 2004, explained the characteristics of Bacillus bacteria which are generally cream-white with irregular colony shapes. According to Hatmati in 2000, he explained that Bacillus sp. has various and uneven colony edges, rough surfaces, is not slimy, and includes dry and powdery. These bacterial colonies are large and do not look shiny. These bacteria are gram-positive bacteria that are expected to grow during the fermentation process, especially shrimp paste fermentation.

In isolating bacteria, it requires various materials such as material samples, a common medium for the growth of Bacillus sp bacteria, a medium for protein hydrolyses such as skimmed milk agar, indole testing (tryptone broth), nutrients from gelatin, sugar, a Medium for starch hydrolysis: Starch Agar, Carbohydrate fermentation media such as lactose solution, glucose solution, sucrose solution, and arabinose solution, Medium for testing methyl red and Voges Proskauer such as Methyl Red solution, medium for nitrate reduction, medium for testing the use of citrate, gram paint, spore paint, bromthymol blue indicator and methyl red indicator, reagents for reducing nitrate using (sulfanilic acid, dimethyl -naphthylamine), reagents for the Voges Proskauer test using (KOH 40% and -naphthol), reagents for indole testing using Kovac's, 3 HgClsolution 2%, alcohol, vaseline, immersion oil, and the last one is distilled water.

After preparing the materials, the next thing to do is to prepare the following tools, clean and sterile sample bottles, water heater, autoclave, incubator, microscope and accessories (it is recommended to use a microscope equipped with complete lenses and lamps for optimal lighting so, the bacterial object is visible.), oven, thermometer, Durham tube, test tube, petri dish, measuring cup, Erlenmeyer, magnifying glass, stirrer, concave glass object in the middle and also the cover glass, Bunsen ose, dropper, and the last is a measuring pipette.

To isolate, the first thing to do is to isolate the bacteria that has been provided. Sampling was done randomly at different locations. In the source that describes the isolation of Bacillus bacteria, sampling was carried out in the city of Semarang in 3 different locations. Each location is divided into several plots which are then assigned a number from each plot. From each plot, the sample will be put into a sterile sample bottle.

The sample that had been put into the bottle will then be given approximately 225 ml of distilled water and then shaken for 10 minutes until the sample settles. After settling, the sample will be poured into an Erlenmeyer and heated to a temperature of approximately 80˚C for 10 – 30 minutes (Brock, 1984).

The sample was made with a very dilute dilution so that the colonies that would grow later were scattered so that the isolation of bacteria would be easier. As much as 1 ml of the sample dilution will be inoculated into a petri dish that is already available. The medium of nutrient agar that has been thawed will be poured into a petri dish of approximately 12 ml. After that, it was carefully homogenized so that the dilution sample was evenly mixed in the medium. Then the next process is incubation at 37˚C & 50˚C for approximately 48-72 hours.

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After that, observations were made on the growth of colonies on each surface in the medium. Each colony that looks different is isolated using a ose needle which will later be scratched on nutrient slanted medium and after that, it will be incubated at 37˚C & 50˚C for approximately 48 hours (mesophilic and thermophilic incubation).

If the growth of Bacillus sp bacteria is seen, each colony is carried out to isolate it from one nutrient sloping medium to another and incubate it at the same time and at the same temperature. This continues until pure isolates are obtained. After obtaining pure isolates, new isolates were formed on a nutrient-slanted medium and stored for isolate stock. After the isolation is complete, the morphological observations of bacterial cell colonies and biochemical tests will be carried out, this aims to obtain the characteristics of the bacteria that appear during isolation. Usually, the observed morphology is the color of the bacteria, the shape, the surface and the diameter of the colony, the state of the edges, the gram, and so on.

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3.1.6 STAPHYLOCOCCUS EPIDERMIDIS

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Staphylococcus epidermidis is one of the pathogenic bacteria that have opportunistic properties or can infect humans who have weak immunity. According to (Chessa et al. 2016), in (Namvar et al. 2014) One of the most common bacteria that can cause disease in humans originating from processed fishery products is Staphylococcus epidermidis. Staphylococcus epidermidis is a bacterium that lives as a parasite in humans and warm-blooded animals. Epidermal staphylococci are most commonly found in the human body and can cause infection when the human immune system is weak (Becker, Heilmann, & Peters 2014).

Staphylococcus epidermidis has a spherical shape with a diameter of approximately 1µm and is also spread in an irregular group. Colonies are round in shape with a smooth, embossed texture and are glossy and have a gray to white color. These bacteria can produce catalase, hyaluronidase and staphylokinase enzymes (Jawetz et al., 2010).

In a study entitled Isolation and Identification of Staphylococcus epidermidis Bacteria in Pinekuhe Smoked Fish which isolated and identified bacteria using smoked fish media, it was stated that the tools used were incubator (YCO-N01), micropipette (Dummo), magnetic stirrer (Wina Type 206).), water bath (Nesco), microscope (Motic Tipe DMB01), laminary flow (Panasonic), dan juga autoclave (Midnif). Dan bahan yang digunakan untuk analisis yaitu BHI Broth (Merck), mannitol salt agar (MSA) (Merck), Sim Agar (Merck), nutrien Agar (Himedia), brain heart infusion broth (Himedia), purple carbohydrate broth (Merck), NaCl 0,9%, koagulase plasma with EDTA (Merck), akuades, hidrogen peroksida (H2O2) 3%, lugol, crystal violet, dan juga safranin (Merck).

Weight the sample of Pinekuhe smoked fish as much as 10 grams, then put in 90 mL of sterile 0.9% NaCl solution and homogenize using a blender for approximately 3 to 5 minutes. After that, 1 mL of suspension was formed with a dilution level of 10-1 and put into 9 mL of sterile 0.9% NaCl solution and homogenized by shaking the tube with a dilution level of 10-2. dilution level of 10-2, and after that, it was put back into 9 mL of sterile 0.9% NaCl solution and homogenized again, this was continued for the next dilution. Take 1 mL of suspension from each diluted sample and transfer it to MSA (Mannitol Salt Agar) media which has previously been labeled with the type of sample and also the level of dilution. After that, the bacterial suspension will be spread using a sterile glass spreader rod evenly over the entire surface of the media and the petri dish will be rotated slowly. After that, the Petri dishes were placed in the incubator and incubated at 37oC for 24 to 48

hours. After the colonies grew on MSA media, they were taken using an Ose needle, then inoculated into BHI broth and incubated for 24 hours at 37oC. after that, it was scraped back onto MSA media and incubated again for 18 to 24 hours at 37oC. Colonies that had grown on MSA media suspected of being Staphylococcus were then selected with the Ose needle transferred to the slant medium by immersing the tip of the OSE needle below the surface until it was tilted and slowly pulled to the tip until a soursop line was formed on the surface of the SA medium. The SA culture was then stored at 4^{o}C.

3.1.7 PEDIOCOCCUS SP

Pediococcus is a genus of bacteria that belongs to lactic acid bacteria (LAB), with non-motile characteristics and has a spherical shape. Bacterial cells are also divided into 2 planes to form pairs, tetrads (composed of four), or larger clumps of spherical cells. Bacteria are gram-positive, spherical in shape, and pairs. The genus Pediococcus belongs to the facultative anaerobic group and requires an environment that is rich in nutrients and also contains growth factors and fermentable genes. Bacteria are also homofermentative and cannot use pentoses (C5-atom carbohydrates) (Victoria, 2008).

The optimum temperature for the growth of Pediococcus is in the range of 25°C - 30°C and the optimum pH is in the range of ± 6 (Victoria, 2008). Several strains of Pediococcus have been identified that have one or more plasmids of various sizes, some of which encode genes for carbohydrate fermentation and also bacteriocin production (Hui, 1994).

Pediococcus acidilactici F-11 is a homofermentative lactic acid bacterium that produces bacteriocins that have been isolated from fermentation products. These bacteria can preserve food because they can produce lactic acid which can lower the pH of the media and then produce bacteriocins (pediocins), so that their presence is faster to suppress the growth of other bacteria. The bacterium P. acidilactici F-11 has been used for the manufacture of salted fish and is also able to suppress the growth of coliform bacteria while also increasing the content of lactic acid bacteria (Nendisa and Rahayu 2001).

Lactic acid bacteria were isolated from the digestive tract of shrimp using a dilution technique and sown on an MRS medium. The preparation of shrimp samples was carried out by first sterilizing the body surface using 0.1% benzalkonium chloride to eliminate the existing bacteria. Then the shrimp was sliced from the dorsal to the anus with a sterile knife, the intestine was taken with sterile tweezers. Then the contents of the law are placed into a petri dish and then washed with sterile distilled water. Intestines that have been weighed 1 gram, then crushed and suspended in sterile dilution water. For dilution, use sterile seawater and distilled water in a ratio between 70% and 30%. Then 10-1,10-2, 10-3, and 10-4 dilutions were carried out. In dilutions of 10-1 to 10-4, it was grown using the pour plate technique in 20 mL of MRS so that 1% CaCO3 and 0.01% Na azide were added. Incubation can be carried out aerobically for approximately 24-48 hours at 37°C. The isolates that produced a clear zone were then grown in MRSB and then transferred to slanted media for preparation for the next stage (Todorov and Dicks, 2004).

Morphologically, lactic acid bacteria can be observed macroscopically or with the naked eye, and microscopically can be observed using a microscope. Visually, the characteristics of colony shape, elevation, edge shape, internal structure, growth on sloping media, and motility could be observed.

In microscopic, we can observe lactic acid bacteria such as cell shape, and cell arrangement and can be observed using a microscope at 1000x magnification.Biochemically it can be done by Gram staining. Gram

staining can be performed on 24-hour-old bacterial cultures which are then grown on solid MRS media. Lactic acid bacteria are Gram-positive bacteria. Gram-positive bacteria will give a purple color when given Gram stain. The purple color can occur because the bacterial cell wall binds to crystal violet paint (Gram A) which is then strengthened by iodine (Gram B) and the crystal violet will not disappear when given the bleaching paint (Gram C), so it is not affected when given the paint. cover (Gram D) which is red. The results of gram staining can also be used to see the shape and arrangement of lactic acid bacteria cells. Observations can be made at 1000x magnification. The results of observations in the microscope obtained are bacteria with rods and cocci or round shapes. While the composition of the cells is mostly in chains and also clustered.

The catalase test was carried out by dripping < 2 drops of 3% H2O2 on a culture aged 24 hours. The positive reaction of the catalase test can be shown by forming bubbles which means there is the formation of Oxygen gas (O2), this is the result of the breakdown of H2O2 by the catalase enzyme produced by these bacteria. Lactic acid bacteria are also catalase-negative bacteria so the results of the catalase test reaction do not form air bubbles and which means no gas is formed.

Fermentation type test is used to classify lactic acid bacteria, between homofermentative or heterofermentative groups. This activity was carried out by growing bacterial cultures on liquid MRS in a test tube containing a Durham tube. Incubation was carried out for 2 days at 37°C. Observations were made by looking at the formation of air bubbles in the Durham tube.

A Carbohydrate fermentation test was carried out using carbon sources such as glucose, D-saccharose, D-galactose, D-sorbitol, glycerol, D-fructose, D-mannose, and D-lactose. Each 5gr/L carbon source was then mixed with MRSB. Then put into a test tube containing a Durham tube of 8 ml. then the isolates aged 24 hours were put into the tube and then incubated at 37°C for 1 day.

Physiological characteristics in this study aimed to observe the growth of lactic acid bacteria isolates against differences in temperature and pH. Temperature affects the growth of isolates, producing bacteriocins can be done by incubating bacteria in MRS Broth at temperatures of 4°C, 10°C, 30°C, 35°C, 40°C, 45°C, 50°C, and 37° C for 24 hours. Growth can be indicated by the presence of turbidity on the Broth MRS media.

The effect of pH on isolates can be done by growing isolates in MRSB with variations in pH starting from 2, 4, 6, 8,10 with the addition of 1 N NaOH or 1 N HCL and then incubated for 24 hours at 37°C. Growth was indicated by the presence of turbidity on MRS Broth media.

The effect of isolates that produce bacteriocin on salt content, using isolates was grown in MRSB with varying levels; NaCl 5%, 8%, 10%, 18% for 24 days. Observations on bacterial growth can be done by looking at the visible or visible turbidity.

4. MARINE FISHERIES FERMENTATION PRODUCTS

4.1. SHRIMP PASTE

Shrimp paste is one of the traditional processed fishery products that are often found in Southeast Asia. This shrimp paste is in the form of a paste, the processing process of which involves salting and fermentation processes. In addition to pasta, shrimp paste can also be found in solid form with a slightly rough texture and has a very distinctive aroma, and sharp, but has a savory taste (Pierson, 2013). Fermentation in shrimp paste is caused by various enzyme activities in the body of shrimp or fish as raw material for shrimp paste (Liviawaty, 2005). Shrimp paste is generally made from small shrimp or known as rebon and can also be made from small fish or anchovies. In various Indonesian food preparations, this shrimp paste is used as a spice or flavoring which adds a distinctive taste.

Shrimp paste is one of the processed fishery products whose manufacturing process uses the fermentation method. As we often see, the shape of the shrimp paste is usually solid with a slightly rough texture and has a very distinctive aroma, and sharp, but savory taste (Pierson, 2013).

According to Adawiyah (2007), shrimp paste that has a good quality value will be dark in color, textured not too soft, and not too rough. Shrimp paste is a processed fishery product made from shrimp. The hallmark of shrimp paste is its red-brown color. The color that arises is influenced by the pigment in the shrimp which is the raw material. This pigment, called astaxanthin, is found in the shell of the shrimp. In addition, other pigments such as astaxanthin also give a distinctive red color to shrimp paste. According to Suprapti (2002), shrimp paste contains fat, protein, carbohydrates, phosphorus, calcium, minerals, water, and iron. In addition, shrimp paste also contains vitamin B12 and amino acids. But basically, the quality of each shrimp paste in the form of texture, aroma, etc. is influenced by the duration of the fermentation process. The longer the time in the fermentation process, the higher the quality of the shrimp paste.

Several types of microbes found in shrimp paste after going through fermentation by adding salt are Micrococcus. The other bacteria, namely Bacillus, Achromobacter, Pseudomonas, and Flavobacterium were found in small amounts at the end of fermentation. And the bacteria isolated from shrimp paste include Micrococcus, Corynebacterium, Bacillus, and Cytophaga.

4.2. LACTID ACIDS FERMENTED PRODUCT AS BEKASAM

Bekasam is a fermented product that is commonly found in Central Java, South Sumatra, and South Kalimantan, the manufacturing process is still spontaneous with freshwater fish, salt, and rice ingredients, you can also use tape. The fermentation time can be around 4-10 days. contain lactic acid bacteria (Desniar, et al., 2011). Commonly used freshwater fish such as catfish (Clarias batrachus), carp (Cyprinus carpio), Tawes fish (Punctius javanicus). During the fermentation process, carbohydrate sources are broken down into simple sugars and converted back into acids and alcohols resulting in decreased product pH which can inhibit the growth of microorganisms and increase total acid during fermentation. The source of carbohydrates is obtained from rice which can be used by Lactic Acid Bacteria (LAB) as an energy source, these bacteria are a group of bacteria that can convert carbohydrates into lactic acid, besides that these bacteria break down carbohydrates into simpler compounds such as propionic acid, ethyl alcohol, and acetic acid. According to Anwar et al (2014), the increase in total lactic acid bacteria in fermentation is influenced by the addition of salt which can stimulate the growth of lactic acid bacteria.

The important role of LAB in fermentation is that it can affect the texture and aroma with the result of increasing the shelf life of the final product (Hugas, 1998). Durability because LAB is related to the process to inhibit the growth of spoilage bacteria and pathogens, inhibition occurs when LAB can produce metabolites such as lactic acid, acetic acid (organic acid), hydrogen peroxide, bacteriocin, and diacetyl (Ross, et al., 2001; Diop). , et al., 2007; Galves. 2007). An organic acid is a preservative commonly used for food because it preserves food, is anti-bacterial, and also acts as a sour taste enhancer. LAB which produces antimicrobials can be used for microbiological food safety culture.

Changes in the texture of the meat in the former are influenced by the duration of fermentation, after being fermented the texture changes to easily crumble, soft, and look pale, soft meat indicates a successful fermentation. The characteristics of successful tamarind are fresh red color, soft flesh, pH between 6.0-6.44, has a sour taste due to the fermentation process (Irianto and Wahyu, 2004).

4.2. FISH SAUCE

Fermented fish sauce is one of several fermented products whose main raw material is fish. Fish sauce is a clear brown liquid, the color is the result of salting fish and usually using salt substitutes as a flavor enhancer in various types of processed foods. The process of making fish sauce fermentation is by mixing fish and salt using a ratio of 1:2 or 1:3 (Lopetcharat et al., 2001).

The process of making fish sauce varies, it takes a long time (4-12 months) (Desniar, 2004) and 6-12 months or even more at room temperature concentrations (30-40ºC) (Lopetcharat et al., 2001). The process of making fish sauce fermentation that takes a long time is by adding enzymes using papain, ficin, and bromelain (Basmal, 1974), viscera and koji (Dissaraphong et al., 2005), and lactic acid bacteria (Tilarsih, 2008). Several other studies have been conducted and show that adding external enzymes such as bromelain and papain can shorten the fermentation time (Gildberg, 1993 and Haard et al., 1994 in BBRPKP, 2010). The process of making fish sauce has two ways, namely spontaneously and by adding a starter (Prasetyo et al., 2012).

The addition of a salt concentration of 20% used in the fermentation process helps inhibit the growth of spoilage microbes and can stimulate the growth of lactic acid bacteria, lactic acid produced from the metabolic process can help lower the pH. Lactic acid activity is one of the factors that has a very important role in the process of making fish sauce fermentation. Lactic acid bacteria can produce bacteriocin compounds that show bactericidal activity against pathogenic bacteria (Purwaningsih et al. 2012; Kusmarwati et al. 2014). There are several types of lactic acid bacteria present in several fermented products such as Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus acidophilus, and there are also several bacteria that have a cocci shape, such as Streptococcus cremoris, Streptococcus thermophillus, Pediococcus pentosaceus, Streptococcus lactis (Yanti et al. 2013; Kusmarwati et al. 2014; Novianti 2013). Muchtadi (2010) said that lactic acid has the characteristics of gram-positive rods and cocci that do not have spores, and catalase-negative.

4.3 SPICY FISH

Spicy fish is a fermented product made from fish. Spicy fish from Indonesia namely Peda. Usually, the fish used to make peda is mackerel (Rastrelliger sp). Peda includes fermented products that are left in a semi-wet state or no further drying process is carried out. This results in the fermentation process in peda fish continuing. Usually, the fermentation that occurs in making fish peda is spontaneous fermentation. In the manufacture of peda fish, additional microbes are not given in the form of starters, but microbes that play an active role during fermentation reproduce spontaneously. This is because the living environment of microbes is suitable to support their growth.

Peda fish fermentation process which is usually carried out by the Indonesian people is by adding salt and this salting is done twice with the salt concentration used generally as much as 25%. Peda fermentation occurs because of the addition of salt which has a function in selecting microorganisms that produce proteolytic enzymes. In this fermentation process, proteolytic enzymes are broken down into simpler molecules, especially glutamic acid (Thariq et al., 2014).

The concentration of salt used in the process of making peda is very influential on the final result of fermentation. High levels of salt are associated with a decrease in water content. This is because when salt enters the fish's body, the water will come out. This process causes changes in the taste, texture, and aroma of the fish. Meanwhile, in this fermentation process, the fat content in peda fish has increased. This is thought to be the result of a decrease in water content in the fish's body (Rahayu et al., 1992 in Desniar et al., 2009). Unlike the case with the protein content in peda fish which has decreased. The use of salt causes the protein in fish meat to dissolve where salt has hygroscopic properties and includes strong electrolytes that can break water molecular bonds and change the natural properties of proteins (Zaitsev et al., in Desniar et al., 2009).

In addition to salting, the process of making peda can also be done by adding fermenter bacteria. These bacteria can be obtained from fermentation products containing lactic acid bacteria such as shrimp paste. In the process of making peda fish, lactic acid bacteria act as antimicrobials that can suppress the growth of spoilage bacteria and can improve the taste of fish (Yunianti et al., 2014). The addition of a starter of lactic acid bacteria in the form of pure isolates in peda fermentation will increase the nutritional quality, aroma, texture, and taste of peda fish (Irianto, 2012 in Yunianti et al., 2014). LAB can produce lactic acid and acetaldehyde compounds in their metabolic processes. Acetaldehyde and lactic acid compounds play a role in shaping the taste and are anti-microbial substances that can suppress the growth of spoilage bacteria (Firmansyah, 209 in Yunianti et al., 2014).

4.4 JAMBAL ROTI SLATED FISH

Jambal Roti is one of the fermented processed fish products originating from Indonesia such as the Pangandaran and Cirebon areas. Jambal roti is one of the product of salted fish. Jambal roti is usually made from Manyung fish (Arius thallassinus). in the journal N. Indriati, 2006 stated that jambal roti is a processed product name given to one type of salted fish. In another explanation, this jambal roti is given to the processed fish because the processed fish is fried, and the texture of the meat will be soft or brittle like baked bread. In general, jambal roti is made by adding salt to 30% of the weight of the fish, and then there are lactic acid bacteria that play a role in it, namely Lactobacillus vitulinus and P. damsonus.

In processed jambal roti, the quality is influenced by the concentration of salt used and also how long the fermentation time is used. Due to the time and amount of salt used, affects the concentration of salt in Jambal Roti, the longer the salting or fermentation process, the salt content of Jambal Roti will increase as well. In Pangandaran, in general, the making of Jambal Roti goes through several processes, namely receiving or preparing raw materials, cutting and cleaning the head and belly of the fish, washing 1, salting about 25% - 35% of the weight of the whole fish, dividing the fish, washing II, drying to dry. while smeared with a solution of sugar and garlic, storage. This jambal roti has a specific flavor and odor and a texture similar to sand (M Karyantina et al, 2021).

CONCLUSION.

The fish fermentation process, which is a biological process based on existing principles, it can be divided into 4 groups, namely: Fermentation with high levels of salt, such as fish sauce, shrimp paste and peda fish. Fermentation with organic acids, such as: making fish silage by adding propionic and formic acids. Fermentation with mineral acids, such as: making fish silage using strong acids. Fermentation with bacteria, such as making lime and Chao anchovies. Some bacteria that can be isolated from marine fisheries in the fermentation process are Bacillus sp; Flavobacterium sp; Halobacterium sp; Corynebacterium sp; Micrococci; and Straphylococci.

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