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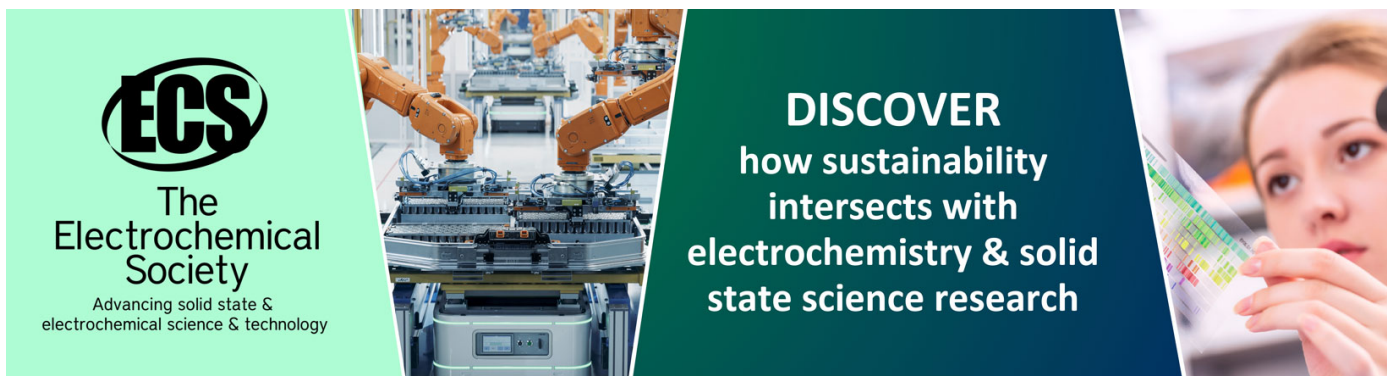
Isolation and identification of bacteria in gastrointestinal of eel (*Anguilla bicolor*) that has potential as probiotic

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Isolation and identification of bacteria in gastrointestinal of eel (*Anguilla bicolor*) that has potential as probiotic

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Abstract. Antibiotic usage in aquaculture serves for the prevention and treatment of diseases but causes an accumulation of residues, and the development of resistant bacterial strains that demands the use of probiotics is increasing. The quality of fish not only seen on the weight of fish but also of bacteria associated with the digestive tract. Some types of bacteria found in the digestive tract of animals have an essential role in order to improve the utilization of feed, fish health, and improvement of environmental quality and microorganisms. The research aims to determine the type of bacteria that can be isolated and characterized from the gastrointestinal tract *Anguilla bicolor* and potential as a candidate probiotic bacteria. This study used data analysis with descriptively. The gastrointestinal tract is the intestine isolation and characterization of bacterial strains obtained. The observations made include morphological characters, hydrolysis tests (fat, starch, protein), and identification of each isolate. From the digestive tract, *Anguilla bicolor* found seven bacterial isolates based on the identification and competent to hydrolyze protein, fat, and starch consists of 3 species thought to be used as probiotics *Anguilla bicolor* are *Bacillus subtilis*, *Bifidobacterium* and *Lactobacillus plantarum*.

1. Introduction

Eel has a high economic value and global demand is increasing. One species that has high economic value is *Anguilla bicolor*. Eel is also used as an export commodity to meet export needs to Japan as a food raw material [1].

Unlike in other countries (Japan and European countries), eel fish resources have not been widely used in Indonesia. This can be seen from the level of utilization of eel fish locally (domestically) is still very low, even though the number of these fish both in seed size and consumption size is quite abundant. One of the reasons is that this fish is not widely known, so that most Indonesian people are not familiar with consuming eel. Similarly, the use of fish for export purposes is still very limited [2].

Various intensification activities of eel fish culture have been developed, but pathogen attack is the main problem in these activities. Diseases in fish culture are a biological risk that must always be anticipated. This encourages the application of integrated and sustainable health management in fish farming.

These probiotic bacteria also provide benefits for the health of living bodies by increasing growth [3,4], milk and egg production and enhancing the immune system which is very useful for the immune system. The concept of using microbes in aquaculture aside from being a probiotic is also considered as a biological control method (biocontrol), which is to eliminate or limit pathogenic microbes to grow



and develop by providing antagonistic microbes. Bacteria as biological controllers are very specific [5]. This is supported by several studies that prove that, using local isolate bacteria for a purpose, gives more effective results than commercial products that generally come from abroad [6,7].

Based on this, identification is needed regarding the type of probiotic bacteria candidates in the digestive tract of eel as a strategy for manipulating microorganisms in preventing disease infections caused by pathogenic bacteria.

2. Materials and methods

2.1. Identification of the type of bacteria

Identification of pure bacteria were identified according to protocol of Amin et al [8] with some modifications. In brief, each bacterium was identified based on morphological characters which included colony morphology and gram tests. Observation of colony morphology seen from the shape, color, and edges of bacterial colonies. Furthermore, each isolate was characterized by biochemical analysis which included fermentation of carbohydrates (glucose, lactose, sucrose, arabinose, mannitol, inositol, maltose), catalase, oxidase, indole, motility, urea, MR-VP, TSIA.

2.2. Carbohydrate fermentation test

Bacterial isolates were inoculated into test tubes containing glucose, lactose, sucrose, arabinose, mannitol, inositol, maltose fermentation medium and then incubated at room temperature for 24 hours. If the bacteria in each of these media change color to yellow, then the result is positive because it forms acid from glucose fermentation and if there is no change in color it is said to be negative.

2.3. Motility test and indole production

Bacterial isolates were inserted into MIO media then incubated for 24 hours at room temperature. For positive motility tests marked by the spread of the colony around the puncture. Furthermore, the medium was dripped with Kovac liquid, the positive indole test was characterized by the presence of a red ring on the surface of the medium.

2.4. Catalase and oxidase

To determine the presence of catalase, it was tested using 3% hydrogen peroxide (H_2O_2) solution in a separate colony. Then 1 drop of H_2O_2 solution is dripped on the surface of the colony. In positive catalase bacteria, there is a formation of gas bubbles around the colony. The oxidase test is done by applying a single colony with a toothpick on the Oxidase Test Strip. The reaction is positive oxidation if the colony's color changes within two minutes to dark blue

2.5. MR-VP test

Bacteria that grow on the media to be inoculated into a test tube filled with MR-VP media then incubated at room temperature for 24-48 hours. The next step is to add 5 drops of the methyl red indicator to the tube. A positive test if the tube is red which means acid and a negative test if the tube is yellow and produce bases. For the VP test by dropping the α naphtol reagent and 40% KOH, the positive test is marked by a red color change, if it is yellow or bronze then the result is negative.

2.6. Casein, fat, and starch hydrolysis test

The isolate was inoculated on MRS media so that each of them would be given skim milk, starch and olive oil. Bacteria that can hydrolyze starch are characterized by the presence of a clear zone around the colony in the test results. The type of bacteria that can hydrolyze proteins is evidenced by the presence of clear zones around the colony in the test results. Bacteria with the ability to hydrolyze fat will cause a yellowish red color at the bottom and around the colony.

3. Results and discussion

Based on research conducted on bacteria found in the digestive tract (intestine) of eel, 8 isolates were obtained based on differences in morphology of bacterial colonies. Identification of each isolate was carried out based on biochemical tests whose results can be seen in Table 1.

From the results of identification, found that bacteria associated with the digestive tract of eel have similar characteristics with *Bacillus sp.*, *Bacillus subtilis*, *Alcaligenes faecalis*, *Lactobacillus plantarum*, and *Bifidobacterium* (Table 2).

In observing the results of casein hydrolysis test is characterized by the presence of clear zones around the isolates. Furthermore, fat hydrolysis is shown by the presence of bright green in the area around the isolate colonies and starch hydrolysis is characterized by the presence of clear yellow zones around the colonies of the isolates that are grown. Bacteria that can hydrolyze casein, starch and fat are with isolate code 1A, 1C, 3C and 1D.

From the observations it can be seen that all isolates show that they can hydrolyze casein which means that these isolates have the activity of protease enzymes which break down proteins into amino acids [8]. Protein hydrolysis occurs due to enzymatic reactions. Bacteria hydrolyze various proteins into a single amino acid with the aim of using these amino acids for the synthesis of proteins and other cellular molecules or as a source of energy [10]. This protein degradation can be used to find out whether bacteria has the potential to assist the digestion of eel, which is mainly in the form of protein in the digestive tract. The bacteria are able to hydrolyze starch because it can produce the enzyme amylase which breaks down flour into maltose. The ability to hydrolyze starch to glucose, maltose, and dextrin because it has the amylase enzyme. Starch cannot be used immediately, so bacteria must hydrolyze starch first into simple molecules and enter cells [9]. The three isolates are able to hydrolyze fat because it can produce lipase enzymes which break down fat molecules into fatty acids and glycerol. According to Gaman, et al [9] fat is a mixture of triglycerides consisting of 1 glycerol molecule that binds with 3 molecules of fatty acids.

Table 1. Biochemical Assay results Gastrointestinal Fish Eel (*Anguilla bicolor*)

characteristics	1.A <i>Bacillus subtilis</i>	2.A <i>Alcaligenes faecalis</i>	3.A <i>Bacillus sp.</i>	1B <i>Bacillus sp.</i>	1.C <i>Bacillus subtilis</i>	3.C <i>Lactobacillus plantarum</i>	1.D <i>Bifidobacterium</i>	3.d <i>Bacillus sp.</i>
test gram	+	-	+	+	+	-	+	+
oxidase	+	-	-	+	+	+	+	-
catalase	+	+	+	+	+	-	+	+
O / F	NR	O	NR	NR	F	O	NR	NR
TSIA	A A	K / K	A A	K / K	A A	A A	A A	A A
LIA	+	+	+	+	+	+	-	+
motility	M	M	NM	NM	M	M	M	NM
indole	-	-	-	+	-	-	+	-
MR	-	-	-	-	+	-	-	+
Vp	-	-	-	-	-	-	-	-
citrate	-	+	-	-	-	+	-	-
urease	+	+	-	+	+	-	-	-
Glucose	-	-	-	-	-	+	-	-
lactose	+	-	+	-	-	+	-	+

sucrose	+	-	-	-	-	+	-	+
arabinose	+	-	-	-	-	+	-	-
mannitol	+	-	-	-	-	+	-	-
inositol	-	-	-	-	+	-	-	-
maltose	+	-	+	-	-	+	-	+

Bacillus subtilis is found in gut samples of 1A and 1C, and can hydrolyze casein, starch, and fat. *Bacillus subtilis* is a rod-shaped bacterium that is Gram-positive [10]. *Bacillus subtilis* has been carried out research related to its role in aquaculture both as a probiotic that inhibits the growth of pathogenic and probiotic bacteria in the digestive process [11]. *Bacillus subtilis* produces bacteriocin which is an antimicrobial substance in the form of polypeptides, proteins, or protein-like compounds. Bacteriocin is synthesized from bosom by bacteria during its growth period and generally only inhibits the growth of bacterial strains that are closely related to bacteriocin-producing bacteria [12]. The addition of *Bacillus subtilis* to the tilapia fish feed can improve the intestinal immune system and increase the survival rate of the fish so that it can increase the bodyweight of the fish.

Bifidobacterium sp. found in a fish intestine sample that is 1D sample and shows that this bacterial colony can hydrolyze casein, starch, and fat. Some characteristics of these bacteria are Gram-positive, anaerobic, not motile, do not form spores, and are rod-shaped [13]. According to Holt *et al.* [14] *Bifidobacterium* sp. gram-positive, rounded colonies, many variations of stem cells arranged in the form of V, sometimes showing a large round shape, negative catalase, and positive oxidase, not motile, fermentative, active fermenting carbohydrates. *Bifidobacterium* habitat is also present in the intestinal system of warm-blooded animals and the intestinal system of honey bees. *Bifidobacterium* bacteria are very effective against harmful bacteria or pathogens that enter from outside as well as harmful bacteria in the digestive tract such as *Shigella dysenteria*, *Salmonella typhosa*, *Staphylococcus aureus*, *E. coli*, and other bacteria. Because these bacteria produce substances that are short-chain fatty acids, especially acetic acid and lactate, and can also produce antibiotic substances.

Lactobacillus plantarum is found in intestinal samples, 3C samples and shows that these colonies can hydrolyze casein, starch and fat. The characteristics of this bacterium are genus of gram-positive, facultative anaerobes. The color of the colony is milky white or beige, the shape of the colony is round, not motile, positive occasase, negative catalase, positive methyl-red, and grows optimally at a temperature of 30-37°C. According to Holt *et al.* [14], these *Lactobacillus* bacteria are gram-positive, nonporous, and not motile by peritrichous, facultative anaerobic flagellates. *Lactobacillus plantarum* including lactic acid bacteria that can inhibit the contamination of spoilage microorganisms, pathogenic microorganisms, and toxin-producing microorganisms, will die. The probiotic candidate bacteria obtained are possible from the eel aquaculture environment. The bacteria enter the fish's digestive tract together with the food eaten. Research by adding *Bacillus subtilis* to feed showed an increase in weight and feed efficiency in Rainbow trout [15].

4. Conclusion

Based on the results of the study, there are 3 types of bacteria associated with the digestive tract of eel (*Anguilla bicolor*) and can be used as a candidate for probiotics, namely *Bacillus subtilis*, *Bifidobacterium*, and *Lactobacillus plantarum*. Further screening tests are needed, namely in vitro inhibition tests, to inhibit the growth of pathogenic bacteria, fungi, and viruses as well as in vivo pathogenicity tests in order to know the benefits in the field of fisheries.

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