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Screening of Amylolytic Bacteria from the Intestines of Tilapia (*Oreochromis niloticus*) Cultivated in Rice Fish Farming in Panembangan Village, Cilongok District, Banyumas

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Abstract

Amylolytic bacteria in the digestive tract of tilapia are bacteria that produce amylase enzymes, which function to break down carbohydrates, thereby aiding the digestive process. The presence of amylolytic bacteria is influenced by feed and the environment. The purpose of this study was to determine the proportion and activity index of amylolytic bacteria in the intestines of tilapia. This study used an observational method with purposive sampling. Amylolytic bacteria were isolated from the intestines of tilapia in Rice fish farming Blocks A and E. The successfully isolated bacteria were grown on 1% starch agar medium with an incubation time of 48 hours and observed at 24-hour intervals. Then, several characteristic tests were carried out, including Gram staining, KOH, catalase, and oxidase tests. Based on the results of the study, the proportion of amylolytic bacteria was found in 10 isolates with a proportion of 44% and 12 isolates with a proportion of 48% in the intestines of tilapia from the two blocks. The average amylolytic activity index obtained from block A was 0.96 ± 0.237, and from block E was 1.49 ± 0.501. The largest amylolytic activity index was obtained in isolate KSA 19 with a value of 1.33 from block A, while from block E, the largest amylolytic activity index value was 2.6 in isolate KSE 5.

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Introduction

A cultivation system that combines the agricultural and fisheries sectors, or fish farming in rice fields alongside rice cultivation, is known as rice fish farming (Ahmadian *et al.*, 2021; Lucas & Southgate, 2003). Compared to monoculture systems, rice fish farming can increase economic income in Indonesia, as demonstrated by farmers in the Sukoharjo region, who experienced a 33.74% increase in profit (total fishery production of 469 kg) (Lestari *et al.*, 2019). Because the rice fish farming system produces two commodities: rice and cultivated fish.

Another rice fish farming area developed in Central Java province, besides the Sukoharjo area, is the Kridoyuwono Rice

Fish Farming Area in Panembangan Village, Banyumas, with tilapia as the leading commodity. Tilapia has many advantages, including fast reproduction, fast growth, and ease of cultivation (Eka, 2020; Diansari *et al.*, 2013). Tilapia are omnivorous, consuming both plant and animal feed for survival (Tesfahun & Temesgen, 2018). The type of feed consumed by fish influences the activity and presence of bacteria in the digestive tract, due to the relationship between the presence of bacteria and the feed consumed (Li *et al.*, 2014; Ringø *et al.*, 2006).

Bacteria found in the digestive tract are both beneficial and harmful (pathogenic). One role of beneficial bacteria is their ability to secrete enzymes to aid in the process of food digestion (Nurhafid *et al.*, 2021; Ray *et al.*, 2012). One of the bacteria that plays a role in digestion is an amylolytic bacterium. Amylolytic bacteria can secrete the enzyme amylase to break down or hydrolyze starch into simpler compounds (Sjofjan & Ardyati, 2011). The main factors that influence the proportion and activity of amylolytic bacteria include the environment and the host (Butt & Volkoff, 2019; Gopinath *et al.*, 2017). According to research conducted by Mondal *et al.* (2008), enzyme-producing bacteria that are high in proportion or almost dominant in the intestines of tilapia are amylolytic and proteolytic bacteria. Screening of amylolytic bacteria was conducted by Putra & Widanarni (2015), who obtained 41 isolates with the highest amylolytic activity index of 20.7 mm in the digestive tract of tilapia. Batubara & Mardhiah (2013) also obtained 12 amylolytic bacteria from 59 isolates, resulting in a proportion of 20.3% for amylolytic bacteria.

Amylolytic bacteria in the intestinal tract of tilapia cultivated in rice fish farming ponds in Panembangan Village, Cilongok District, Banyumas Regency are unknown. This study was conducted to determine the proportion and amylolytic activity index of bacteria in the digestive tract of tilapia in rice fish farming ponds in Panembangan Village.

Materials and methods

Time and Place of Research

This research was conducted from September to October 2022. Fish sampling was conducted at the Kridoyuwono Rice Fish Farming Group in Panembangan Village, Cilongok District. The amylolytic test was conducted at the Research Laboratory of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University, and the Microbiology Laboratory of Muhammadiyah University of Purwokerto.

Fish Sampling

Fish intestines sampling was performed surgically. First, the tilapia samples were killed by piercing the head. Then, the tilapia were weighed with a digital scale to determine the total weight, and the total length was measured using a millimeter block. Fish intestines sampling was carried out by dissecting the abdomen and then carefully removing the intestines using tweezers. The intestines were placed on a millimeter block to measure their total length. Then, the fish intestines were cut into 5 cm lengths for each fish, and then ground with a mortar and pestle. Empty microtubes were weighed and prepared aseptically. Then, 0.2 g of the ground intestines was placed into the same microtube and homogenized using 1 mL of physiological solution.

Isolation of Bacteria

The initial procedure for bacterial isolation involves dilution in stages. Each sample was serially diluted using five test tubes containing 4.5 mL of sterile physiological solution (dilution 10^{-1}-10^{-5}). 0.5 mL of the sample was taken from the tube and placed into the first test tube containing 4.5 mL of physiological solution. The test tube was homogenized to obtain the first dilution (10^{-1} dilution). A total of 0.5 mL of the sample suspension was taken from the first tube and homogenized in the second tube (10^{-2} dilution), and this procedure was carried out up to the fifth tube (10^{-5} dilution). Samples from dilutions 10^{-2} to 10^{-5} were taken as much as 5 mL each and cultured using the pour plate method in sterile petri dishes. Next, the warm sterile liquid tryptone soya agar (TSA) Somedium was poured into the petri dish containing the dilution results and homogenized, then the samples were incubated for 24 hours at 28°C. Based on the formula, the bacterial colonies that grew were counted using the total plate count (TPC) method (Madigan & Martinko 2006).

$$\text{Number of bacteria (CFU/g)} = \text{Number of colonies} \times \frac{1}{\text{Dilution}} \times \frac{1}{\text{Culture volume}} \times \frac{1}{\text{Sample weight}}$$

The next step is bacterial screening. According to Cappucino & Welsh (2019), bacterial screening involves isolating colonies based on size, shape, margin, elevation, and color. Each appearance of the morphology of the bacterial colony that differs in each petri dish from the dilution results is taken to be recultured on new TSA media. The number of colonies transferred is 50 from the total sample. After that, the dish containing the isolate is stored as stock.

Amylolytic Activity Test

Bacteria that have been screened based on morphology and re-cultured for stock are then tested for amylolytic activity. Bacterial isolates are grown by streaking on

specific starch media or 1% starch and incubated for 24-48 hours at 28ºC. The clear zone formed by amylolytic bacteria after incubation is measured at 15 total diameter, and the colony diameter is monitored by adding 1% iodine solution to facilitate visualization.

The resulting clear zone was measured using a formula for calculating amylolytic activity as quantitative data. The calculation formula refers to the formula used by Zubaidah *et al.* (2019):

$$\text{Amylolic activity index} = \frac{\text{clear zone diameter}}{\text{bacterial colony diameter}}$$

The proportion of amylolytic bacteria was calculated using the formula of Sinatryani *et al.*, (2014):

$$\text{Proportion of amylolytic bacteria (\%)} = \frac{\text{Number of amylolytic bacterial colonies obtained}}{\text{Total number of colonies observed}} \times 100$$

Gram KOH Test

The Gram KOH test aims to determine the Gram type of a bacterium. A Gram test is performed by placing 1-2 drops of 3% KOH (potassium hydroxide) solution onto a glass object, following which a loop of the bacterial isolate from the purification results is placed on a glass object containing 3% KOH solution and mixed thoroughly. The results of the Gram KOH test are characterized by a thick and sticky texture, indicating that the bacterial colony is Gram-negative, but if it is not thick, the bacterial colony is Gram-positive (Hardiansyah *et al.*, 2020). The percentage of Gram-positive and Gram-negative results is calculated using the formula:

$$\text{Gram positive percentage (\%)} = \frac{\text{Total number of gram} - \text{positive colonies}}{\text{Total number of colonies observed}} x\ 100$$

$$\text{Gram negative percentage(\%)} = \frac{\text{Total number of gram} - \text{negative colonies}}{\text{Total number of colonies observed}} x\ 100$$

Catalase Test

The catalase test is conducted to determine catalase activity in bacteria by adding a 3% H_2O_2 solution. One drop of the 3% H_2O_2 solution is placed on a glass slide. The bacterial isolate is taken using a needle and then a thin line is drawn on the glass slide containing the H_2O_2 solution. Test results marked with the presence of bubbles indicate a positive catalase test, while results without bubbles indicate a negative catalase test (Begum *et al.*, 2017).

Oxidase Test

The oxidase test is performed by taking an isolate using a loop needle and then scratching it on a glass slide. After that, the cut filter paper is placed on top of the isolate and dipped in 1% Tetramethyl-phenylenediamine dihydrochloride. The oxidase test is considered positive if the filter paper turns blue to black, and negative if there is no color change (Cappucino & Welsh, 2019).

Data Analysis

The data obtained from the research included bacterial abundance, colony morphology, gram proportion, amylolytic bacterial activity index, and amylolytic bacterial proportion. These data were presented in tables and documentation figures, then analyzed descriptively.

Results and Discussion

Number of Bacteria in the Intestinal Tract of Tilapia Fish

The total number of bacteria in the intestines of tilapia fish can be determined by calculating using the total plate count (TPC) method. The results of the calculation of the number of bacteria in each block were 2.82 x 10^6 CFU/g for fish cultivated in block A, and 1.66 x 10^5 CFU/g for fish cultivated in block E. Therefore, it can be seen from the results obtained that the abundance of bacteria in the intestines of tilapia fish cultivated in rice fish farming ponds is still within normal limits or still within the range of previous research.

Research on the total number of bacteria in the intestines of tilapia has also been conducted, including by Valenzuela-Armenta *et al.* (2018), who found bacterial counts ranging from 4.0 x 10^1 to 9.8 x 10^5 CFU/g. According to research by Al-Harbi and Uddin (2004), the bacterial abundance ranged from 8.9 x 10^5 to 1.3 x 10^9 CFU/g in the intestines of tilapia. According to research by Sugita *et al.* (1997), the total bacterial count in the intestines of freshwater fish ranged from 10^3 to 10^8 CFU/g, which included aerobic, facultative anaerobic, and obligate anaerobic bacteria. The abundance of bacteria in the digestive tract of fish varies from individual to individual. The main factors influencing the high or low abundance of bacteria in the digestive tract of fish are intrinsic and extrinsic (Butt & Volkoff, 2019).

Intrinsic factors are factors originating from within the host, such as species, age, weight, and immune system. Extrinsic factors are factors originating from outside or from the environment, namely water quality parameters including pH, temperature, salinity, and antibiotics (Talwar *et al.*, 2018). Bacteria originating from the aquatic environment can enter the mouth along with water or food, then pass through and/or reside in the digestive tract (Austin, 2006). Bacteria that only pass through the digestive tract with food are called allochthonous bacteria, while the group of bacteria that reside in the fish's digestive tract and can associate with the host's tissue is called autochthonous (Ringø *et al.*, 1995).

Morphology of Intestinal Bacterial Colonies in Tilapia Fish

Table 1. Morphology of Bacterial Colonies from the Intestines of Tilapia Fish Cultivated in Rice Fish Farming Ponds in Panembangan Village, Cilongok (Block A)

Isolate Code	Morphology of Intestinal Bacterial Colonies of Tilapia Block A			
	Shape	Elevation	Edge	Colour
KSA1	Irregular	Convex	Entire	Milky white
KSA2	Irregular	Convex	Entire	Cream white
KSA3	Circular	Convex	Entire	Clear white
KSA4	Circular	Pulvinate	Entire	Milk white
KSA5	Circular	Convex	Entire	Milk white
KSA6	Circular	Umbonate	Entire	Milky white
KSA7	Circular	Flat	Entire	Creamy white
KSA8	Circular	Flat	Entire	Clear white
KSA9	Circular	Flat	Entire	Yellow
KSA10	Rhizoid	Flat	Lobate	Clear white
KSA11	Rhizoid	Flat	Filamentous	Clear white
KSA12	Circular	Umbonate	Entire	Milk white
KSA13	Filamentous	Convex	Filamentous	Creamy white
KSA14	Irregular	Convex	Entire	Yellow
KSA15	Irregular	Raised	Lobate	Yellow
KSA16	Circular	Pulvinate	Entire	Thick white
KSA17	Circular	Flat	Entire	Yellow
KSA18	Irregular	Pulvinate	Entire	Thick white
KSA19	Filamentous	Flat	Filamentous	Clear white
KSA20	Irregular	Convex	Lobate	Clear white
KSA21	Circular	Umbonate	Entire	Thick white
KSA22	Circular	Umbonate	Filamentous	Clear white
KSA23	Filamentous	Pulvinate	Filamentous	Thick white
KSA24	Spindle	Flat	Entire	Cream
KSA25	Circular	Pulvinate	Entire	Yellow

Colony morphology observation is a step taken to observe the colonies formed macroscopically from the incubation results to determine the bacterial characteristics and differentiate bacterial types. Bacterial characteristics that can be identified include shape, elevation, size, margins, and color (Cappucino & Welsh, 2019). The bacterial isolates obtained were 25 isolates for block A and 25 isolates for block E. Morphological characteristics are shown in Tables 1 and 2.

The results of the observation of various bacterial colony morphologies were obtained in the intestines of tilapia raised in rice fish farming ponds, blocks A and E. There were colonies in spindle, circular, irregular, filamentous, and rhizoid shapes. The dominant elevation was convex and flat, and the edges formed were mostly entire.

The resulting color varied from white to milky white, yellow, and deep yellow. Differences in colony morphology characteristics in the isolates obtained will be used in the screening process for amylolytic bacteria used to identify and characterize bacteria (Mamou *et al.*, 2016). Several factors influencing these morphological differences are the type of bacteria, bacterial

physiology, and culture media substrate (Sousa *et al.*, 2013).

Based on the results obtained from the Gram test on 25 bacterial isolates from each blocks, in block A, there were 20 Gram-negative bacterial isolates with a proportion of 80% while Gram-positive isolates obtained a proportion of 20% with the remaining 5 bacterial isolates. In block E, the proportion of Gram-negative bacteria was 44% with 11 isolates, and for the proportion of Gram-positive bacteria, the results were 56% with 14 bacterial isolates.

Table 2. Morphology of Bacterial Colonies from the Intestines of Tilapia Fish Cultivated in Rice Fish Farming Ponds in Panembangan Village, Cilongok (Block E)

Isolate Code	Morphology of Intestinal Bacterial Colonies of Tilapia Block E			
	Shape	**Elevation**	**Edge**	**Colour**
KSE1	Circular	Flat	Entire	Milky white
KSE2	Circular	Convex	Entire	Cream white
KSE3	Circular	Flat	Entire	Cream white
KSE4	Circular	Flat	Entire	Cream white
KSE5	Punctiform	Flat	Entire	Clear white
KSE6	Irregular	Raised	Undulate	Clear white
KSE7	Circular	Pulvinate	Entire	Milk white
KSE8	Circular	Pulvinate	Entire	Cream
KSE9	Spindle	Convex	Entire	Cream
KSE10	Rhizoid	Flat	Lobate	Clear white
KSE11	Irregular	Flat	Undulate	Clear white
KSE12	Irregular	Flat	Undulate	Clear white
KSE13	Circular	Flat	Entire	Yellow
KSE14	Circular	Pulvinate	Entire	Yellow
KSE15	Irregular	Raised	Undulate	Clear white
KSE16	Irregular	Raised	Lobate	Clear white
KSE17	Irregular	Flat	Lobate	Clear white
KSE18	Circular	Pulvinate	Entire	Cream white
KSE19	Circular	Convex	Entire	Thick white
KSE20	Circular	Raised	Entire	Cream white
KSE21	Circular	Convex	Entire	Milk white
KSE22	Circular	Flat	Entire	Cream white
KSE23	Circular	Convex	Entire	Cream white
KSE24	Circular	Raised	Entire	Cream white
KSE25	Circular	Convex	Entire	Yellow

Gram KOH Test of Bacteria from Tilapia Fish Intestines

The Gram test is performed to determine or identify whether the bacterial isolate obtained is Gram-negative or Gram-positive using 3% KOH (potassium hydroxide) reagent. Gram-negative bacteria are indicated by the presence of mucus, while Gram-positive bacteria do not produce mucus.

The proportion of Gram bacteria obtained in Figure 1 shows that the highest

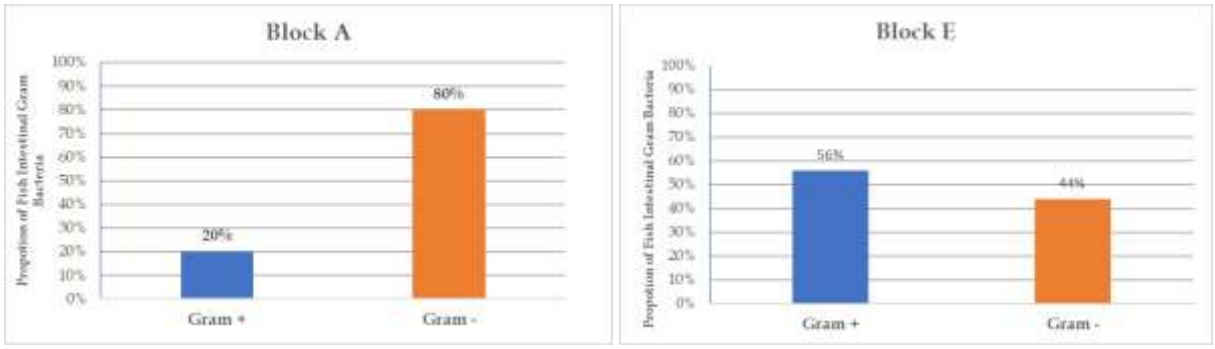

Figure 1. Gram Proportion of Bacteria from the Intestines of Tilapia Fish Cultivated in Rice Fish Farming Ponds in Panembangan Village, Cilongok

proportion of Gram-negative bacteria is in block A tilapia. Meanwhile, in block E, the proportion of Gram-positive bacteria was higher than that of Gram-negative bacteria. The difference in the proportion of Gram-negative bacteria obtained between these two blocks is thought to be influenced by the presence of bacteria in the cultivation environment that enter the digestive tract through feed and water. This results in the diversity of bacteria in the environment being similar to that inhabiting the fish's digestive tract (Cahill, 1990).

The high proportion of Gram-positive bacteria in the intestines of tilapia fish cultivated in block E ponds is suspected to contain organic materials from anthropogenic activities in the surrounding environment and other environmental factors that can increase the population of bacteria that can decompose organic compounds into inorganic ones as nutrients for the soil (Adithiya *et al.,* 2017). According to the research of Adithiya *et al.,* (2017) and Fadilah *et al.,* (2022) obtained Gram-positive bacteria as decomposers of organic materials in waters, some of these bacteria came from the genus *Bacillus,* but this genus of bacteria is not only from waters, but can also be found naturally in fish organs such as the liver and digestive tract.

Proportion of Amylolytic Bacteria

Amylolytic bacteria are bacteria that produce amylase enzyme, characterized by a clear zone surrounding the isolate in starch solutions. Isolating amylolytic bacteria using starch-enriched media allows us to determine the number or proportion of amylolytic bacterial isolates and their activity based on the amylolytic bacterial proportion graph in Figure 2.

The results of the proportion of amylolytic bacteria obtained from the intestines of tilapia fish cultivated in block A

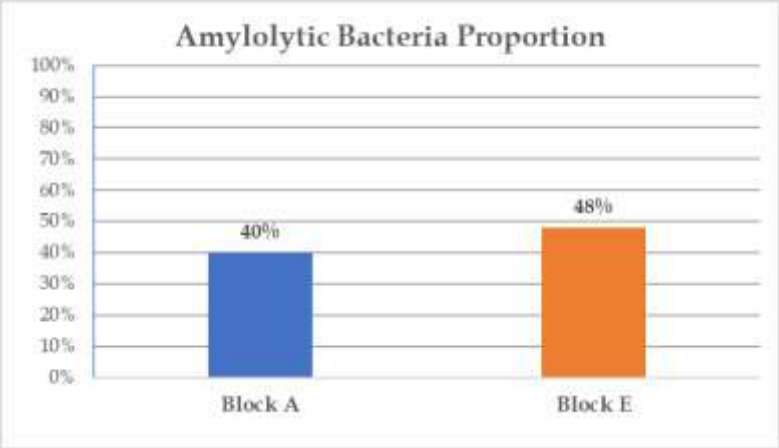

Figure 2. Proportion of Amylolytic Bacteria from the Intestines of Tilapia Fish Cultivated in Rice Fish Farming Ponds in Panembangan Village, Cilongok

were 40% with 10 of the total 25 isolates tested amylolytic. Block E showed a percentage of 48% with a total of 12 isolates from 25 isolates tested amylolytic. The higher proportion of amylolytic bacteria obtained in block E was influenced by the pond water, because in pond E, it is suspected that there is more organic material from anthropogenic activities in the surrounding environment. The higher the organic material contained in the water media, the faster the growth of bacteria contained in the media (Putra *et al.,* 2014).

Another factor thought to influence the proportion of amylolytic bacteria in the fish intestine is the feed consumed. The proportion obtained almost reached half of the total isolates tested for amylolytic activity. This is thought to be because tilapia are omnivorous fish with herbivorous tendencies. Previous research reported that bacteria isolated from tilapia intestines obtained a proportion of amylolytic bacteria of around 23% - 20.3%, obtaining 12 isolates out of 59 isolates (Batubara & Mardhiah, 2013). Research on other omnivorous fish has also been conducted by Rahmawati (2020), who found 52 amylolytic isolates out of 75 total isolates, with a proportion of 69%. Marlida & Elrifadah (2017) obtained 9 isolates out of a total of 12 bacterial isolates, with a proportion of 75%. According to (Listiowati *et al.,* 2022), in the herbivorous Nilem fish, the amylolytic proportion was 43%, with 3 amylolytic isolates out of a total of 7 isolates. Due to their consumption of plants with fiber or carbohydrates, omnivorous fish also have a proportion of amylolytic bacteria in their digestive system in addition to herbivorous fish.

The carbohydrate source for tilapia cultivated in the Panembangan Village Rice Fish Farming Area is pellet feed, which contains carbohydrates from wheat and bran. Other carbohydrate sources include sente leaves, which contain 22% starch (Melisha *et al.*, 2016). This feed serves as a substrate for bacterial growth and development, indicating that these feeding habits influence the proportion of amylolytic bacteria in the fish's intestines (Mondal *et al.*, 2008; Sumathi & Priya, 2011).

Amylolytic Activity Index

Amylolytic bacterial activity can be observed, and its activity index can be measured if a clear zone (halo zone) has formed around the bacterial colonies growing on starch media after incubation. This clear zone is formed from the hydrolysis or breakdown of starch polymers by the amylase 24 enzyme secreted by the bacteria (Hanzen *et al.*, 2017). Observation of the clear zone in amylolytic bacteria can be facilitated by dripping iodine solution onto the media. Hydrolyzed starch cannot bind with iodine (I2), resulting in a clear zone around the bacterial colonies. Starch media that have not been or are not hydrolyzed will be dark blue due to the bond between the starch and iodine (Putri *et al.*, 2021).

Figure 3 shows that all bacteria were able to grow on starch agar, but some isolates did not produce clear zones. This is because these bacterial isolates do not produce the amylase enzyme that can hydrolyze starch and instead utilize the nutrients from starch agar for growth and metabolism (Basitoh *et al.*, 2018).

The results of the calculation of the amylolytic bacterial activity index obtained from both blocks can be seen in Figure 4 and Appendix 4, which shows that the amylolytic

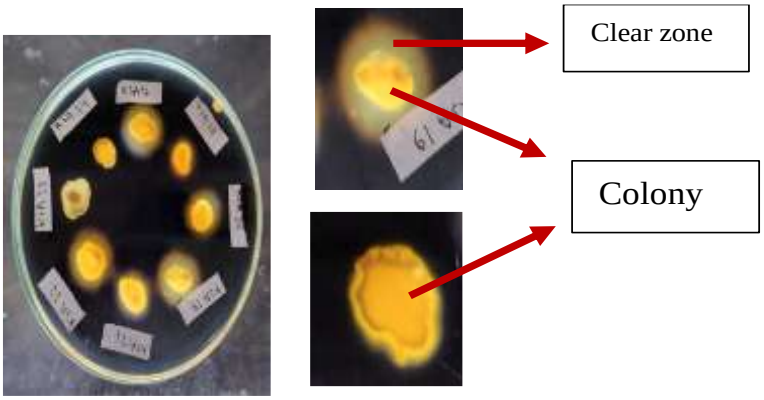

Figure 3. Clear Zone Results of Amylolytic Bacterial Activity Tests from the Intestines of Tilapia Fish Cultivated in Rice Fish Farming Ponds in Panembangan Village, Cilongok.

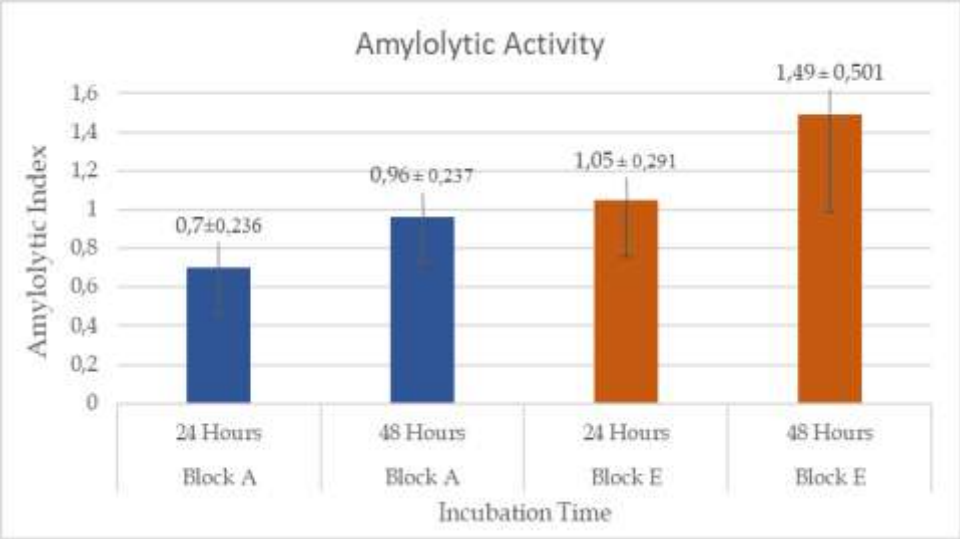

Figure 4. Amylolytic Activity Index of Bacteria from the Intestines of Tilapia Fish Cultivated in Rice Fish Farming Ponds in Panembangan Village, Cilongok

bacterial activity from block A at a 24-hour incubation interval obtained the highest activity value of 1.00, and the lowest was 0.16. Meanwhile, at a 48-hour interval, the highest value was 1.33 and the lowest was 0.60. The amylolytic activity index in block E for a 24-hour incubation interval obtained the highest value of 1.76 and the lowest value of 0.17. Meanwhile, at a 48-hour interval, the highest value was 2.6 and the lowest was 0.6. The average obtained from the results of the index calculations for both blocks at a 24-hour interval was 0.70 in block A and 1.05 in block E. Meanwhile, the average index for both blocks at a 48-hour interval was 0.96 for block A and 1.49 for block E.

According to Dar *et al.* (2015), the amylolytic index can be categorized into three levels. The amylolytic activity index is categorized as high if the value is ≥3, the moderate category is ≥1 – 2.9, and the low category is categorized as low if the value is 0-0.9. Based on this, the amylolytic index value obtained from each isolate, almost all isolates from both blocks are included in the moderate category because the highest value only reached 2.6, which was owned by the isolate code KSE 5. However, several isolates with amylolytic activity were categorized as low, with the lowest value of 0.16 in KSA 21. The difference in the amylolytic index value produced by each bacterial isolate depends on the ability of each bacterium to hydrolyze starch by producing 27 extracellular amylase enzymes, which are influenced by internal factors in the form of bacterial types (Hastuti

et al., 2017). External factors during the isolation of amylolytic bacteria are from the culture environment or culture media, such as pH, temperature, incubation time, and growth media substrate (Mulyani *et al.,* 2018; Ningtyas *et al.,* 2023; SenGupta *et al.,* 2012).

Based on the amylolytic index table, isolates KSA 11 and KSE 19 had decreased in amylolytic activity index after 48 hours of incubation. The amylolytic index obtained in isolate KSA11 was 0.79 after a 24-hour incubation time, and 0.6 after 48 hours of incubation. Isolate KSE 19 also experienced a decrease in its amylolytic index, where at a 24-hour incubation time, the index value was obtained at 1.50, but after 48 hours, it became 1.40. This can be obtained because the area of the clear zone diameter in the media is still the same or does not increase in area, while the area of the bacterial colony diameter increases after a 48-hour incubation time. The effect of the length of incubation time is related to the availability of nutrients in the media on the activity of bacteria producing enzymes. The longer the incubation period, the fewer nutrients are present in the media as bacterial growth increases, and in this condition, the activity of bacteria in producing enzymes will decrease (Susilawati *et al.,* 2015). Amylolytic activity reaches its maximum limit in the stationary phase. The stationary phase is the metabolic phase of bacterial defense for life, in this phase growth slows and the growth rate is equal to the death rate or it can be said that in this phase the number of bacterial cells does not increase because the number of growing cells is equal to the number of dead cells, this is caused by reduced nutrient levels and the accumulation of metabolic waste. Additionally, bacteria also produce many secondary metabolites, including enzymes, to maintain their lives during this phase (Hasanah *et al.,* 2020; Melisha *et al.,* 2016).

Catalase and Oxidase Test

The catalase test is a test conducted to determine whether a bacterium can produce the catalase enzyme. The catalase enzyme is activated by 3% hydrogen peroxide (H_2O_2). Bacteria that produce catalase positively will form bubbles on the isolate that is dripped with H_2O_2, while catalase-negative bacteria do not produce bubbles. These bubbles are produced from the decomposition of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) (Yulvizar, 2013). The oxidase test is a test conducted with the aim of determining the presence of the oxidase enzyme in a bacterium. Bacteria that are streaked on filter paper are dripped with 1% tetramethyl-phenylene diamine hydrochloride. Bacteria that have the oxidase

Table 3. Results of Catalase and Oxidase Tests of Amylolytic Bacteria from the Intestines of Tilapia Fish Cultivated in Rice Fish Farming Ponds in Panembangan Village, Cilongok.

Isolate Code	Test Results	
	Catalase Test	Oxidase Test
KSA 2	-	-
KSA 11	+	+
KSA 15	+	+
KSA 16	-	+
KSA 17	+	+
KSA 18	-	+
KSA 19	+	+
KSA 20	-	+
KSA 21	-	+
KSA 22	-	+
KSE 1	+	+
KSE 2	-	+
KSE 3	-	-
KSE 4	-	+
KSE 5	-	+
KSE 6	-	-
KSE 11	-	-
KSE 18	-	+
KSE 19	-	+
KSE 20	-	+

enzyme or are oxidase positive are indicated by the appearance of a dark blue color on the filter paper after the reagent is dripped. Meanwhile, bacteria that do not have the oxidase enzyme will not produce a color (Cappucino & Welsh, 2019).

Based on the test results in Table 3, four bacterial isolates from block A and one bacterial isolate from block E contained the catalase enzyme. The oxidase test for both blocks found that the bacteria contained more oxidase enzymes, while a few bacteria did not. The catalase enzyme in these bacteria functions to break down hydrogen peroxide (H_2O_2), a metabolic product that is toxic to the bacterial cells themselves. The oxidase enzyme plays a role in the oxidation and electron reduction processes (Anggraini *et al.*, 2016).

These two tests can help in characterizing bacteria because each genus and species of bacteria has diverse characteristics such as morphology, Gram-positive properties, and the ability to produce

catalase or oxidase enzymes. Research by Ginting *et al.* (2018) and Novitarizky *et al.* (2018) identified the characteristics of amylolytic bacteria using several tests, including catalase and oxidase tests. The identification results from both studies found characteristics that matched those of *Staphylococcus spp.*, *Micrococcus sp.*, and *Lactobacillus sp*. These four species are capable of producing catalase or oxidase enzymes, but some bacteria do not produce these enzymes.

Characteristics of Amylolytic Bacteria in the Intestines of Tilapia Fish

Based on the results, the bacterial isolates with the highest amylolytic activity were isolates KSA 19 from block A and KSE 5 from block E. The KSA 19 bacterial isolate is a Gram-negative bacterium that has a filamentous morphology with filamentous edges and flat elevations. This isolate also produces catalase and oxidase enzymes. The KSE 5 isolate is a Gram-positive bacterium that has a punctiform colony morphology, with flat elevations and entire edges. The KSE 5 isolate is capable of producing catalase enzymes but not oxidase enzymes.

Conclusion

The proportion of amylolytic bacteria from the intestines of tilapia cultivated in rice fish farming ponds in Panembangan Village, Cilongok, obtained from block A was 40% and from block E was 48%. The amylolytic activity index of bacteria from the intestines of tilapia cultivated in rice fish farming ponds in Panembangan Village, Cilongok, from both blocks had an average of 0.96 ± 0.237 in block A and 1.49 ± 0.501 in block E. The highest amylolytic activity index was from block A, namely 1.33, and from block E, 2.6. Meanwhile, the lowest activity indexes from the two blocks, were block A and block E, namely 0.16 and 0.17.

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