

## Study of the Presence of Streptococcosis in Tilapia Cultivation in Banyumas Regency

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### ABSTRACT

Tilapia is the most widely cultivated fish in Banyumas which is susceptible to bacterial diseases caused by the increasing number of disease cases in tilapia (*Oreochromis niloticus*) due to high cultivation intensification. One of the diseases that often attacks is streptococcosis caused by the bacteria *Streptococcus* sp., which can cause mass mortality and major economic losses for farmers. The purpose of this study was to identify pathogenic bacteria in farmed tilapia in Banyumas. The method used in this study was a purposive sampling method, namely by taking samples of tilapia that have characteristics of disease symptoms such as protruding eyes, damaged fins, and red wounds on the scales from three locations, namely Beji Village, Karangsalam Village, and Pasir Wetan Village. Bacterial samples were obtained from four organs, namely the brain, kidneys, eyes, and liver, then observed the morphology of bacterial colonies and Gram KOH tests were carried out. The results showed that bacterial colonies found in most organs were round to irregular in shape, white to yellowish in color, and Gram positive, which shows the characteristics of *Streptococcus* sp. bacteria. Based on these results, it can be concluded that *Streptococcus* sp. bacteria are still the main threat in tilapia cultivation in Banyumas, so it is necessary to take preventive measures through the implementation of good biosecurity, the use of immunostimulants, and the development of vaccines to maintain the sustainability of tilapia cultivation businesses, especially in Banyumas Regency.

### INTRODUCTION

Central Java ranks third in Indonesia for fish farming, with a total fish production of 458,513.77 tons in 2022. One of the main areas for fish farming in Central Java is Banyumas

Regency, which ranks second in Central Java with a total production of 8,085 tons (Yuliani & Sandri, 2024).

Tilapia is an example of a successfully cultivated fish worldwide, ranking third after salmon and shrimp according to the Department of Fisheries and Aquaculture of the Food and Agriculture Organization (FAO). Tilapia belongs to the phylum Chordata, class Pisces, subclass Teleostei, order Percomorphi, suborder Percoidea, family Cichlidae, genus *Oreochromis*, and species *Oreochromis niloticus* (El-Sayed & Fitzsimmons, 2023). Tilapia (*Oreochromis niloticus*) is one of the most commonly farmed freshwater fish species due to its rapid growth, ability to survive in a wide range of environmental conditions, and high economic value. However, the intensification of high-density cultivation often leads to fish health problems, particularly infectious diseases that can cause significant economic losses. One of the most detrimental diseases is streptococcosis, caused by infection with bacteria of the genus *Streptococcus*, particularly *Streptococcus agalactiae* and *Streptococcus iniae* (Abdallah *et al.*, 2024).

Streptococcal infection in tilapia can cause clinical symptoms such as exophthalmia, histopathological symptoms, internal bleeding, nervous system disorders, and even mass mortality with high mortality rates (Lusiastuti *et al.*, 2016). The impact of this disease on aquaculture is significant, making it a major concern in the global aquaculture industry. Recent research indicates that *S. agalactiae* is the dominant pathogen affecting the success of tilapia production (He *et al.*, 2021). *S. agalactiae* infections occur in various tilapia farming locations in Indonesia, affecting fish of various developmental stages and sizes. Two biotypes have been identified: biotype 1 ( $\beta$ -hemolytic) and biotype 2 (non-hemolytic). The  $\beta$ -hemolytic type has been detected in tilapia ready for consumption and potential broodstock in Papua, South Kalimantan, and Jambi, while the non-hemolytic type has been found in Java and Gorontalo (Suhermanto *et al.*, 2020). *Streptococcus* bacteria are characterized by their spherical or oval shape, resembling elongated chains. They are gram-positive and catalase-negative (Holt *et al.*, 1994). Lusiastuti *et al.*, 2016, also reported that *Streptococcus* bacteria are intolerant to 6.5% salt concentration and negative catalase test.

Various prevention and control measures have been studied, ranging from the use of antibiotics to vaccination. However, antimicrobial resistance to certain antibiotics has begun to be detected in *Streptococcus* isolates from tilapia cultivation, necessitating a more sustainable approach to fish health management (Abu-Elala *et al.*, 2020). Alternative strategies such as biosecurity, immunostimulants, and new technology-based vaccinations continue to be developed to reduce mortality rates and maintain sustainable production (Maulu *et al.*, 2021). The aim of this study was to identify the presence of *Streptococcus* sp. in tilapia in Banyumas Regency.

## METHODS

This research began with sampling tilapia (*Oreochromis niloticus*) farmed in Beji Village, Karangsalam Village, and Pasir Wetan Village. Bacterial isolation and identification were then carried out in the Genetics and Microbiology Laboratory, Faculty of Agriculture and Fisheries, Muhammadiyah University of Purwokerto. This research was conducted from September to October 2025.

### Sampling

This study employed a survey method, with tilapia selected using purposive sampling. This method was chosen because sample selection was based on specific considerations relevant to the research objective, namely to identify fish showing clinical signs of disease or

abnormal conditions during cultivation. Fish samples were taken from three tilapia cultivation sites located in Banyumas Regency: Beji Village, Karangsalam Village, and Pasir Wetan Village. From each location, fish exhibiting clinical symptoms such as protruding eyes and frayed fins were identified. Careful selection of fish was carried out to ensure that the samples accurately reflected the condition of the diseased fish at each site. After collection, each fish sample was placed in a container filled with water from its original pond and then transported to the laboratory for further examination, including isolation and identification of the disease-causing pathogen. This purposive sampling method is expected to reflect the condition of tilapia suspected of being infected with the disease in each cultivation location, thus providing an accurate picture of the disease's presence in tilapia in that region.

### **Bacterial Isolation and Characterization**

Target organ bacterial isolation begins with a stepwise dilution method to reduce the number of microbes in the sample. After selecting the appropriate dilution, the next step is to use the pour plate technique using Tryptic Soy Agar (TSA) media. A diluted bacterial suspension is placed in an empty petri dish, followed by the addition of the still-liquid, warm agar medium (Amanda & Ayuzar, 2016). This mixture is then gently stirred by rotating the petri dish and allowed to solidify. After incubation, the pour plate technique produces colonies distributed both on the surface and within the agar, allowing the selection of a single, isolated colony to obtain a pure culture. These pure cultures were then transferred to fresh agar media for stock maintenance and prepared for further identification.

### **Data Analysis**

Bacterial morphology was observed after the bacteria grew on TSA media. The observed morphology included colony size, colony shape, colony color, elevation, colony margins, and surface properties. Descriptive morphological analysis was performed to align the obtained bacterial colony morphology with characteristics similar to *Streptococcus* sp., supported by the bacterial identification book Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Further identification involved a Gram stain test using KOH. This test involves mixing a loop of a pure colony with a drop of KOH solution on a glass slide. If a slime thread forms when the loop is pulled, the bacteria are classified as Gram-negative, as their cell walls are lysed by KOH. Conversely, if no slime is formed, the bacteria are Gram-positive, due to their thick cell walls being more resistant to strong alkaline solutions (Hardiansyah *et al.*, 2020).

## **RESULTS**

The research results indicate clinical symptoms in tilapia caused by *Streptococcus* sp. bacteria. The investigation included observing clinical symptoms in fish samples from three different villages: Beji, Pasir Wetan, and Karangsalam. This was followed by initial characterization of bacteria isolated from the internal organs of the affected fish. Clinical observations revealed common but also site-specific disease manifestations.

In fish samples taken from Beji Village (Figure 1.A), the main clinical symptoms observed were fin rot and protruding eyes (exophthalmia), with additional specific symptoms including pale corneas. Furthermore, fish samples from Pasir Wetan Village (Figure 1.B) exhibited fin rot, distinguishing them by the presence of red spots on the scales on the underside of the fish's body. The most severe condition was observed in samples from Karangsalam Village (Figure 1.C), which, in addition to fin rot, also had one of the fish's eyes missing.

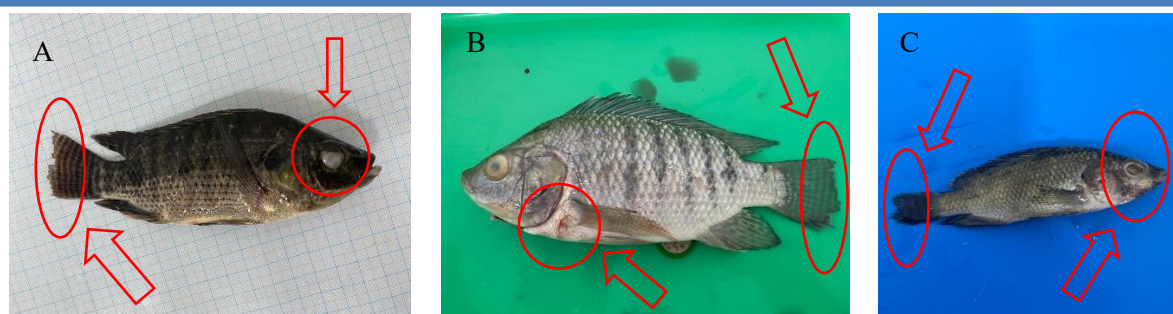


Figure 1. Fish Samples Collected in (A) Beji Village, (B) Pasir Wetan Village, and (C) Karangsalam Village

Overall, the microorganisms sampled displayed a dominant morphology, with white colonies, flat elevations, and smooth surfaces. However, there were clear differences; colonies varied in shape between round and irregular, and sizes ranging from small dots to large. The largest exception in morphology was found in the isolate from the Pasir Wetan eye, which had a rhizoid shape and only appeared in large sizes. It was the only isolate with a rough surface and was also yellow in color. Variations in the colony margins were also observed; although the most common margin was flat, wavy, curled, and grooved margins were also detected in various organs. The results of the Gram-KOH test were very consistent, with all showing positive results, indicating that all isolates studied likely have a uniform cell wall structure.

Table 1. Bacterial Morphology Data and Gram-KOH Test

No	Location	Organ	Size	Form	Color	Elevation	Edge	Surface Properties	Gram KOH
1	Beji	Brain	Punctiform, small	Circular	White, yellow, brown	Flat	Entire, curled	Smooth	+
		Kidney	Large, punctiform, small	Irregular, circular	White	Flat	Entire, undulate	Smooth	+
		Eye	Large, punctiform, small	Irregular, circular	White	Flat	Entire, undulate, lobate	Smooth	+
		Liver	Punctiform, small, large	Irregular, circular	White	Flat	Entire, undulate	Smooth	+
2	Pasir Wetan	Brain	Punctiform, small, large	Irregular, circular	White	Flat	Entire, undulate	Smooth	+
		Kidney	Punctiform, small, large	Irregular, circular	White	Flat	Entire, curled	Smooth	+
		Eye	Large	Rhizoid, irregular	White, yellow	Flat	Undulate	Rough, smooth	+
		Liver	Punctiform, small, large	Irregular, circular	White	Flat, convex	Entire	smooth	+
3	Karangsalam	Brain	Large	Irregular, circular	White	Flat	Entire	Smooth	+
		Kidney	Punctiform, small, large	Irregular, circular	White	Flat	Entire	Smooth	+

No	Location	Organ	Size	Form	Color	Elevation	Edge	Surface Properties	Gram KOH
		Eye	Punctiform, small, large	Irregular, circular	White	Flat, convex	Entire	Smooth	+
		Liver	Punctiform, small, large	Irregular, circular	White	Flat	Entire	Smooth	+

## DISCUSSION

Clinical symptoms observed in tilapia from Banyumas Regency include torn/torn caudal fins, protruding eyes (exophthalmia), missing eyes, and red spots on the scales. These results are very consistent with the manifestation of *Streptococcus* sp. disease in tilapia (*Oreochromis niloticus*). Clinical symptoms that begin to appear include changes in the eye organs such as swelling (exophthalmia), cloudiness (opacity), white eyes (purulence), and lysis of the eyes (Taukhid & Purwaningsih, 2011). According to Utami *et al.* (2013), damage to the physical condition of fish due to *S. iniae* infection includes destroyed fins, loose scales, a body resembling the letter "C", and an enlarged stomach. In addition, symptoms of bleeding (red spots) on the scales and base of the fins are also often reported in cases of septicemia caused by *Streptococcus* sp. The initial diagnosis of this clinical symptom is that it is caused by the bacteria *Streptococcus* sp. Damage to the eyes and fins indicates an advanced infection, often involving internal organs such as the brain and kidneys, consistent with the organs targeted for isolation in this study.

Isolation results demonstrate diverse colony morphology, although predominantly white, flat-topped, and smooth-surfaced. Variations in shape (circular, irregular, rhizoidal), size, and margins (entire, undulate, curled, lobate) within the isolates indicate that more than one bacterial species is infecting the fish or growing on the media. Pathogenic bacteria such as *Aeromonas hydrophila* (Gram-negative) often exhibit round or irregular colonies, and *Streptococcus* sp. (Gram-positive) typically forms small, transparent to white colonies (Admi *et al.*, 2024).

The most notable exception was an isolate from an eye in Pasir Wetan Village, which had a rhizoidal shape, large size, and a rough surface, as well as a yellow color. This rhizoidal morphology and rough surface are not common in *Streptococcus*, the main Gram-negative pathogen in fish, suggesting the possibility of this isolate being a contaminating bacterium or another species, such as *Bacillus* sp. (which can form rhizoidal colonies) or the non-pathogenic *Staphylococcus* group, which sometimes exhibits color variations (Preenanka & Safeena, 2023). According to Yulvizar (2013), the colonies formed were round, with serrated edges, raised colony elevations, and yellow or cream-colored colonies. This variation in morphology emphasizes the need for more in-depth biochemical and molecular identification to determine the dominant pathogen.

The morphological data obtained corresponded to the morphology of *Streptococcus* sp., which exhibits a coccal (round) shape, white color, and Gram-positive bacteria (Daenuri & Sinaga, 2011). The Gram KOH test results showed positive (+) results in all isolates, according to Kurnia *et al.* (2015) who said that gram-positive cells do not create a thick solution or emerging threads. Methodologically, it indicates that all isolates have thin cell walls and are classified as Gram-Negative bacteria. In principle, a positive KOH test (slime formation) occurs due to the lysis of the thin cell walls of Gram-Negative bacteria by the strong base KOH. However, this contradicts the strong clinical suspicion that the disease is caused by

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*Streptococcus* sp., which is taxonomically a Gram-Positive bacteria. Gram-positive cells do not form a thick gel or emerging strings (Hardiansyah *et al.*, 2020).

### CONCLUSION

Based on the observation of clinical symptoms, which include bulging eyes, damaged or peeling fins, red lesions on the scales, and loss of eyes, all of these symptoms are very consistent with the signs of Streptococcosis disease caused by *Streptococcus* sp. bacteria in tilapia (*Oreochromis niloticus*). Findings from the observation of the morphology of bacterial colonies identified in most organs and the results of the Gram KOH test which indicated that all isolates were Gram positive, strengthen the characteristics of the *Streptococcus* sp. type of bacteria. Therefore, it can be concluded that *Streptococcus* sp. bacteria still pose a significant threat to tilapia cultivation efforts in Banyumas Regency, so that preventive measures are needed such as the implementation of adequate biosecurity, the use of immunostimulants, and the development of vaccines so that the sustainability of tilapia cultivation efforts in the area is maintained.

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