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Isolation and Molecular Identification of Pathogenic Bacteria in Giant Gourami (*Osphronemus gouramy*) Cultivated in Tamansari and Singasari Villages, Banyumas Regency

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ABSTRACT

Giant gourami (*Osphronemus gouramy*) is a freshwater fishery commodity with high economic value and is widely cultivated in Banyumas Regency. However, the emergence of diseases caused by pathogenic bacteria often becomes an obstacle in cultivation activities because it can reduce the survival rate and productivity of fish. This study aims to identify the types of pathogenic bacteria found in gouramy fish raised in Tamansari and Singasari Villages, Banyumas Regency. The research method was carried out by taking liver samples of gouramy fish which were then isolated on TSA media. Identification was carried out based on the morphological characteristics of bacterial colonies, the Gram KOH test, and molecular identification. The results showed that from both locations, several bacterial isolates were obtained with similar colony morphological characteristics, namely round (circular), small size, smooth edges, smooth surface, and cream color. The results of the Gram KOH test showed that all isolates were Gram-negative bacteria. Based on these characteristics, the isolates have similarities with the genus Acinetobacter which is often found as an opportunistic bacteria in freshwater fish. The results of molecular identification also showed that the bacteria obtained from the cultivated gouramy fish were *Acinetobacter* sp. This study provides a preliminary overview of the presence of opportunistic bacteria such as *Acinetobacter* sp., which have the potential to become pathogens in gourami when water quality conditions are unstable.

INTRODUCTION

Giant gourami (*Osphronemus gouramy*) is a freshwater fishery commodity with high economic value and is widely cultivated in Indonesia. This high economic value has led many fish farmers to cultivate gourami. Banyumas Regency is one of the gourami producers. Banyumas Regency is known as a productive center for freshwater fish cultivation and is a

regency with a high level of gourami production, producing various products ranging from eggs and fry to consumption-sized fish (Khumaidi & Hidayat, 2018). Gourami cultivation is widely carried out in villages in Banyumas Regency, including Tamansari and Singasari Villages, due to promising domestic market demand. However, a frequent problem is disease caused by bacteria in gourami, which is a barrier to cultivation efforts, causing mass mortality, reduced growth, and significant economic losses (Saraswati *et al*., 2020). According to Rahayu *et al*. (2019), in freshwater fish farming, bacterial diseases that may attack freshwater fish include *Aeromonas hydrophila, A. salmonicida, Pseudomonas anguilliseptica, Streptococcus agalactiae, S. iniae, Vibrio* sp., *Edwardsiella tarda, E. ictaluri, Mycobacterium* sp., *Yersinia* sp., and *Acinetobacter* sp.

Previous research has shown that bacteria such as *Aeromonas hydrophila* and other Aeromonas species are often the main cause of bacterial infections in gourami, such as Motile Aeromonas Septicemia (MAS). Various pathogenic bacteria are known to attack gourami and cause significant economic losses. *Aeromonas hydrophila* is one of the main pathogens frequently found in gourami, causing diseases such as skin ulceration, hemorrhage, and mass mortality (Purbomartono *et al*., 2019). Furthermore, *Aeromonas sobria* has also been reported to cause infections that often occur in conjunction with *Aeromonas hydrophila*, worsening the fish's condition. Infections caused by these bacteria typically occur due to suboptimal cultivation conditions, such as poor water quality and high fish density (Khumaidi & Hidayat, 2018).

In addition to *Aeromonas* sp., *Mycobacterium* sp. is also a concern because it causes the chronic and difficult-to-treat disease Mycobacteriosis. This disease is characterized by the formation of granulomas in the fish's internal organs and can lead to slow death (Rahmaningsih & Yanuar, 2014). The presence of other pathogenic bacteria, such as *Flavobacterium columnare* and *Edwardsiella tarda*, has also been reported to infect gourami, causing various clinical symptoms that are detrimental to fish farmers. Therefore, accurate identification of pathogenic bacteria is crucial for effective disease control.

To isolate and identify pathogenic bacteria in samples, various methods have been developed, from conventional agar culture methods like TSA (Typticase Soy Agar) to molecular methods like PCR (Polymerase Chain Reaction) and 16S rRNA sequencing, which offer advantages in accuracy and speed of identification. Molecular methods can overcome the limitations of the bacterial identification process, which is difficult to culture, and provide genetic data that can be used for phylogenetic and other further studies (Noer, 2021).

Based on this background, this study aims to isolate and molecularly identify pathogenic bacteria that attack gourami fish farmed in Tamansari and Singasari villages, Banyumas Regency. The results are expected to provide information on the possible types of pathogenic bacteria circulating in these farms, thus providing a basis for disease control and increasing the productivity of gourami fish farming in the region.

METHODS

This research was conducted in February-March 2025. The fish samples used in this study were five gourami (*Osphronemus gouramy*) from fish farming ponds in Tamansari and Singasari Villages, Banyumas Regency. The selected fish samples were gourami (*Osphronemus gouramy*) that showed no clinical signs of disease. The sampling method used in this study was purposive random sampling. The fish were transported alive in plastic containers and jerry

cans for sampling and isolation at the Microbiology and Genetics Laboratory, Faculty of Agriculture and Fisheries, Muhammadiyah University of Purwokerto.

The fish were dissected aseptically using sterile surgical scissors and tweezers. The liver was sampled to serve as a source of inoculant. One gram of the liver sample was then weighed and placed into a 1.5 mL microtube. The sample was crushed using a micropestle until smooth, then added with 1 mL of physiological solution and homogenized using a vortex. The homogenized samples were then subjected to a 10^{-1}–10^{-3} dilution process using the serial dilution method (Cappuccino & Welsh, 2018). The 10^{-1}–10^{-3} dilutions used three test tubes filled with 4.5 mL of sterile physiological saline (Nurhafid et al., 2021). Each of the dilution samples was inoculated using the pour plate technique. 1 mL of inoculant was taken and placed into empty, sterile Petri dishes, which were then grown on Typticase Soy Agar (TSA) medium. Warm TSA medium (±40-45°C) was poured into the Petri dishes and homogenized. The plates were then incubated for 24 hours at 28°C to allow bacterial colony growth.

The growing colonies were observed based on their macroscopic morphology, including size, shape, margins, elevation, color, and surface characteristics. Colonies with different morphologies were selected and Gram-stained using the 3% KOH method to differentiate between Gram-positive and Gram-negative bacteria (Agung *et al*., 2024). Isolates exhibiting different morphologies were then re-cultured in Typticase Soy Broth (TSB) media and incubated at 28°C for 24 hours to obtain sufficient cell biomass for DNA extraction.

DNA isolation was performed using the Zymo Research Quick-DNA Miniprep Kit according to the written procedure. 200 µL of bacterial cells cultured in TSB media were taken for DNA analysis, transferred to a microcentrifuge tube, followed by 200 µL of BioFluid & Cell Buffer (Red) and 20 µL of Proteinase K. The homogenized sample was vortexed for 10-15 seconds, and then incubated at 55°C for 10 minutes. After that, lysis was taken by adding 420 µL of Genomic Binding Buffer then vortexed and spun down for 10-15 seconds and the lysis solution was transferred to the Zymo-Spin™ IIC-XLR Column into the Collection Tube which was then centrifuged for 1 minute at 12,000 rpm. The sample was centrifuged for 1 minute at 12,000 rpm Then, 400 µL of DNA Pre-Wash Buffer was added and centrifuged for 1 minute at 12,000 rpm. Add 700 µL of g-DNA Wash Buffer and centrifuged for 1 minute at 12,000 rpm, add 200 µL and centrifuged for 1 minute at 12,000 rpm. The final step is to add 50 µL of DNA Elution Buffer, and store the DNA in a -20°C refrigerator.

16S rRNA gene amplification was performed using universal primers 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R (5’-TACGGYTACCTTGTTACGACTT-3’) using the Polymerase Chain Reaction (PCR) method ordered from PT. Genetika Sciences Indonesia. The PCR procedure used the MyTaq™ HS Red Mix protocol from Bioline. The PCR process consisted of 40 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 15 seconds. The initial denaturation was set at 95°C for 2 minutes and the final extension at 72°C for 5 minutes. PCR results were verified using 1.5% agarose gel electrophoresis. The obtained DNA was then sent to PT. Genetika Sciences Indonesia for sequencing.

Data analysis of bacterial morphological characteristics and gram KOH tests will be carried out by comparing various literature sources including using Bergey's Manual of Determinative Bacteriology. Then the sequencing data obtained from PT. Genetika Sciences was then edited and aligned with ClustalW using the MEGA 12 application. The alignment results were then compared with the GenBank (NCBI) database using BLAST (Basic Local Alignment Search Tool) on the web https://blast.ncbi.nlm.nih.gov/Blast.cgi. Phylogenetic analysis was carried out using MEGA 12 software with the Neighbor-Joining method and 1000

times bootstrap (Kumar *et al*., 2018). Samples were identified based on sequence similarity with reference data in GenBank.

RESULTS

Bacterial isolation using gourami liver as an inoculant using serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3} yielded nine isolates. These isolates were then subjected to colony morphology and subjected to a Gram-positive KOH test. Table 1 shows that most of the morphological characteristics are small, round, convex, with a smooth surface and flat edges, and cream-colored colonies. The Gram-negative KOH test indicated that the bacteria were gram-negative, as indicated by the thickening of the KOH solution (a thin mucus formation).

Table 1. Identification Data for Bacteria Found in Gourami in Tamansari and Singasari Villages, Banyumas Regency

Village	Bacterial Isolates	Gram KOH Test	Morphological Characteristics of Bacterial Colonies					
			Size	Shape	Elevation	Edge	Surface Properties	Color
Tamansari	K.1.1	-	Punctiform Small	Circular Spindle	Flat Convex	Entire	Smooth	Cream
	K.1.2	-	Punctiform Small	Circular Spindle	Flat Convex	Entire	Smooth	Cream
	K.1.3	-	Punctiform	Spindle	Flat	Entire	Smooth	Cream
	K.2.1	-	Punctiform Small	Circular Spindle	Flat Convex	Entire	Smooth	Cream
	K.2.2	-	Punctiform Small	Circular Spindle	Flat Convex	Entire	Smooth	Cream
	K.2.3	-	Punctiform Small	Circular Spindle	Flat Convex	Entire	Smooth	Cream
Singasari	K.3.1	-	Punctiform Small	Circular Spindle	Flat Convex	Entire	Smooth	Cream
	K.3.2	-	Punctiform Small	Circular	Flat	Entire	Smooth	Cream
	K.3.3	-	Punctiform	Circular	Flat	Entire	Smooth	Cream

Molecular analysis results (Table 2) indicate that 16S rRNA gene amplification produced a DNA fragment approximately 1,450 bp in length. BLAST analysis of the gene sequences showed the highest similarity of 93.49–93.57% with several species in the genus Acinetobacter, including *A. pittii*, *A. calcoaceticus*, and *Acinetobacter* sp.

Table 2. Results of Gene Sequencing Analysis Using Basic Local Alignment Search Tools (BLAST) of Bacterial Samples Cultured on TSB Media from Gourami Liver

No	Description	Max Score	Total Score	Query Cover	E value	Ident	Accession
1.	*Acinetobacter* sp.	2013	2013	100%	0.0	93.57%	EU998912.1
2.	*Acinetobacter pittii*	2013	2013	100%	0.0	93.51%	MH144257.1
3.	*Acinetobacter* sp.	2008	2008	100%	0.0	93.49%	MG755253.1
4.	*Acinetobacter* sp.	2008	2008	100%	0.0	93.49%	MW578944.1

No	Description	Max Score	Total Score	Query Cover	E value	Ident	Accession
5.	*Acinetobacter pittii*	2008	2008	100%	0.0	93.49%	MH211299.1
6.	*Acinetobacter calcoaceticus*	2008	2008	100%	0.0	93.49%	MK834827.1
7.	*Acinetobacter pittii*	2008	2008	100%	0.0	93.49%	OP648202.1
8.	*Acinetobacter calcoaceticus*	2008	2008	100%	0.0	93.49%	PV424122.1

A phylogenic tree constructed with MEGA 12 using the Neighbor-Joining method with 1,000 bootstrapping runs showed that the "sample" was closely related to several Acinetobacter species, particularly *A. pittii* and *A. calcoaceticus* (Figure 1). *Aeromonas hydrophila* (NR043638.1), which is far removed from the main Acinetobacter cluster, was used as an outgroup.

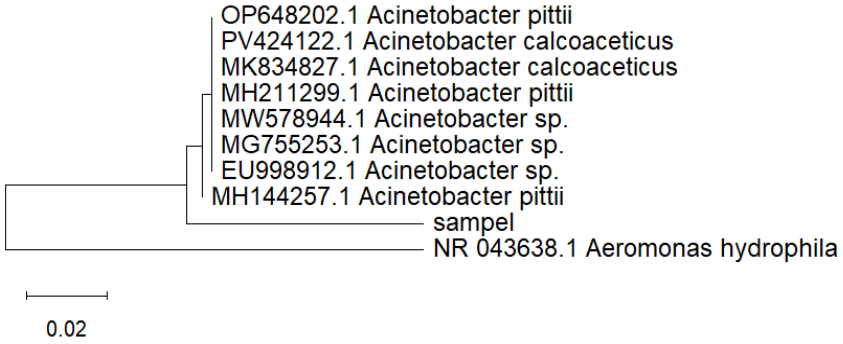

Figure 1. Phylogenetic Tree Resulting from 16S rRNA Gene Analysis of Bacterial Isolates from Gourami Fish in Tamansari and Singasari Villages, Banyumas Regency (Outgroup: NR_043638.1 — *Aeromonas hydrophila*)

DISCUSSION

Observations of the morphological characteristics of bacterial colonies in Table 1 show that all bacterial isolates obtained from gourami (*Osphronemus gouramy*) cultivated in Tamansari and Singasari villages displayed small (punctiform) colonies with a circular or spindle shape, flat to convex elevations, entire edges, smooth surfaces, and a cream color. These characteristics align with the description of the genus Acinetobacter in Bergey's Manual of Determinative Bacteriology (John et al., 1994), which states that Acinetobacter colonies are typically round, have convex elevations, have smooth surfaces, and are unpigmented. Creamy or grayish-white colony color is characteristic of species such as *A. calcoaceticus* and *A. pittii* (Lucidi et al., 2024).

Gram staining using KOH showed that all bacterial isolates were gram-negative. According to Agung et al. (2024), a 3% KOH Gram-stain test performed on gram-negative bacteria will indicate thick, fatty, and thin-walled bacteria. The KOH reacts with the fat and causes the gram-negative cells to rupture, releasing their genetic material (DNA). The long, sticky DNA molecules cause the test results to appear slimy when the loop needle is removed (Figure 2).

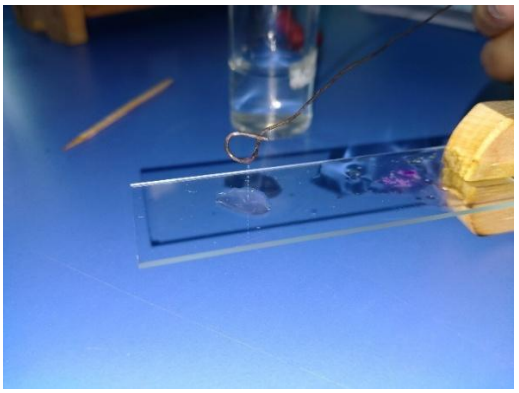

Figure 2. Gram-stain KOH Test Process (Showing the Characteristics of Gram-Negative Bacteria)

16S rRNA gene sequencing analysis using the Basic Local Alignment Search Tool (BLAST) showed the highest similarity level of 93.49%–93.57% with species in the genus Acinetobacter (Table 2), specifically *A. pittii* (MH144257.1, OP648202.1) and *A. calcoaceticus* (MK834827.1, PV424122.1). Although identities below 97% indicate potential local strain variation or new species within the *A. calcoaceticus–A. pittii* complex, these results still confirm a close genetic relationship within the group (Li *et al*., 2017).

The phylogenetic tree (Figure 1), constructed using the neighbor-joining method and a bootstrap count of 1,000, indicates that the "sample" isolates are most closely related to *Acinetobacter pittii* (OP648202.1 and MH211299.1) and *Acinetobacter calcoaceticus* (MK834827.1 and PV424122.1). All these isolates form a monophyletic cluster clearly separated from the outgroup *Aeromonas hydrophila* (NR043638.1). This confirms the BLAST results that the study samples from this group are known as opportunistic bacteria that can trigger disease in fish, especially in high-density aquaculture or under stressful conditions. Phylogenetic analysis also suggests that the isolate from Banyumas may be a local variant of *A. pittii* that has adapted to freshwater aquaculture environments.

Data integration demonstrates consistency between the isolate's phenotypic and genotypic characteristics. *Acinetobacter pittii* is known as an opportunistic bacterium that can infect fish through skin wounds or stress from poor water quality. These data indicate that the isolate from gourami in Banyumas could pose a threat to fish health if left uncontrolled. Preventive measures include pond sanitation management, the use of probiotics, and routine microbiological monitoring.

Overall, these results indicate that the gourami aquaculture systems in Tamansari and Singasari are dominated by bacteria of the genus Acinetobacter. The pond environment, rich in organic matter and poor water circulation, favors the growth of these opportunistic bacteria. Therefore, early identification and control of water microbes are crucial to prevent widespread infection in gourami aquaculture in Banyumas.

CONCLUSION

This study shows that the bacterial isolates found in gourami (*Osphronemus gouramy*) from Tamansari and Singasari Villages, Banyumas Regency, belong to the Acinetobacter pittii group based on colony morphology, Gram-positive and Gram-negative KOH tests, and 16S rRNA gene sequence analysis. The isolates exhibited small, circular, cream-colored colonies, confirming the bacteria's Gram-negative nature. These results align with the description in

Bergey's Manual of Determinative Bacteriology, which states that Acinetobacter are short, non-motile, Gram-negative rods that form smooth, white to cream-colored colonies.

Phylogenetic analysis revealed that the isolates were closely related to *A. pittii* and *A. calcoaceticus*, indicating that the bacteria are opportunistic members of the *Acinetobacter calcoaceticus–pittii* complex. These findings support the hypothesis that *A. pittii* has the potential to cause bacterial infections in gourami, especially under suboptimal cultivation conditions. The analysis provided a preliminary picture of the presence of opportunistic bacteria that could jeopardize aquaculture activities. Therefore, early detection and management of water quality and pond sanitation are essential to prevent the emergence of diseases caused by these bacterial infections and provide a basis for strategies to prevent bacterial infections in freshwater fish farming.

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REFERENCES

Agung, L. A., Hermawan, D., & Herjayanto, M. (2024). Identifikasi Awal Bakteri Kandidat Probiotik Asal Usus ikan Nila dengan Metode Uji Gram Menggunakan KOH 3%. *Leuit : Journal of Local Food Security*, *5*(1), 369–373.

Cappuccino, J. G., & Welsh, C. (2018). *Microbiology A Laboratory Manual* (11th ed.). Pearson Education.

John, G. H., Noel, R. K., Peter, H. A. S., James, T. S., & Stanley, T. W. (1994). *Bergey's Manual of Determinative Bacteriology* (9th ed.). Williams & Wilkins.

Khumaidi, A., & Hidayat, A. (2018). Identifikasi Penyebab Kematian Massal Ikan Gurami (*Osphronemus gouramy*) di Sentra Budidaya Ikan Gurami, Desa Beji, Kecamatan Kedung Banteng, Kabupaten Banyumas, Jawa Tengah. *ResearchGate*, *3*(2), 145–153. https://doi.org/10.31093/joas.v3i2.53

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Li, J., Cao, J., Wang, X., Liu, N., Wang, W., & Luo, Y. (2017). *Acinetobacter pittii*, an emerging new multi-drug resistant fish pathogen isolated from diseased blunt snout bream (*Megalobrama amblycephala* Yih) in China. *Applied Microbiology and Biotechnology*, *101*, 1–13. https://doi.org/10.1007/s00253-017-8392-4

Lucidi, M., Visaggio, D., Migliaccio, A., Capecchi, G., Visca, P., Imperi, F., & Zarrilli, R. (2024). Pathogenicity and virulence of Acinetobacter baumannii: Factors contributing to the fitness in healthcare settings and the infected host. *Virulence*, *15*(1), 2289769. https://doi.org/10.1080/21505594.2023.2289769

Noer, S. (2021). Identifikasi Bakteri secara Molekular Menggunakan 16S rRNA. *EduBiologia Biological Science and Educational Journal*, *1*(1). https://doi.org/10.26539/edubiologia.v1i1.8596

Nurhafid, M., Syakuri, H., Oedjijono, O., Listiowati, E., Ekasanti, A., Nugrayani, D., & Pramono, H. (2021). Isolasi dan Identifikasi Molekuler Bakteri Proteolitik dari Saluran Pencernaan Ikan Nila (*Oreochromis niloticus*) yang Dibudidayakan di Kabupaten Banyumas. *Jurnal Perikanan Universitas Gadjah Mada*, *23*(2), 95–105. https://doi.org/10.22146/jfs.64072

Purbomartono, C., Mulia, D. S., & Priyambodo, D. (2019). Respon Imun Non-spesifik Ikan Gurami (*Osphronemus gouramy*) yang Diberi Fucoidan dari Ekstrak Rumput Laut Cokelat Padina sp. *Sainteks*, *16*(1), 9–17.

Rahayu, N. N., Prayogo, Ulkhaq, M. F., Kenconojati, H., & Azhar, M. H. (2019). Identifikasi Bakteri pada Komoditas Ikan Air Tawar di Balai Karantina Ikan, Pengendalian Mutu dan Keamanan Hasil Perikanan Surabaya I. *Journal of Aquaculture Science*, *4*(2), 102–110.

Rahmaningsih, S., & Yanuar, U. (2014). Zoonotik Bakteri *Mycobacterium tuberculosis* yang Menginfeksi Ikan Gurami (*Osphronemus gouramy* Lac.). *Jurnal Harpodo Borneo*, *7*(1), 36–40.

Saraswati, A. R., Saintika, Y., Thohari, A. N. A., & Iskandar, A. R. (2020). Sistem Pakar Diagnosis Penyakit Ikan Gurami (*Osphronemus gouramy*) Menggunakan Case Based Reasoning. *Jurnal Teknologi Informasi dan Ilmu Komputer*, *7*(4), 779. https://doi.org/10.25126/jtiik.2020701953