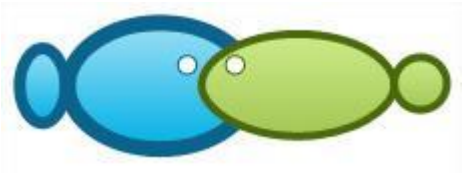

# Molecular identification of pathogenic bacteria isolated from cultured eels (*Anguilla bicolor*) in Notog Village, Banyumas Regency, Indonesia

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**Abstract**. Cultured eel (*Anguilla bicolor*) represents an economically significant aquaculture commodity; however, bacterial infections continue to pose a major challenge by reducing fish health and production performance. The objective of this study was to detect and identify pathogenic bacteria isolated from cultured eels in Notog Village, Patikraja District, Banyumas Regency, Indonesia, through molecular analysis targeting the 16S rRNA gene. Twenty eel samples were collected from a single culture pond, and bacterial isolates were obtained from liver tissues. The isolates were cultured on Tryptone Soya Agar (TSA) and subsequently observed on Glutamate Starch Phenol (GSP) medium. Genomic DNA was extracted from bacterial cultures and amplified using universal bacterial primers 27F and 1492R. PCR products were visualized by agarose gel electrophoresis, sequenced, and analyzed using BLAST against the GenBank database. Phylogenetic relationships were reconstructed using the Neighbor-Joining method with 1000 bootstrap replications in MEGA 12. PCR amplification produced clear DNA bands of approximately 1500 bp in both isolates, consistent with the expected size of the nearly full-length bacterial 16S rRNA gene. BLAST analysis indicated that isolate 1.4.3 exhibited the highest similarity to *Aeromonas dhakensis* (97.19%), while isolate 4.4.3 showed the highest similarity to *Aeromonas veronii* (96.86%). Phylogenetic analysis confirmed that both isolates clustered within the *Aeromonas* clade and were distinctly separated from the outgroup *Streptococcus agalactiae*. These results demonstrate that pathogenic *Aeromonas* species are associated with cultured eels in the study area. The findings underscore the importance of molecular surveillance, water quality management, and biosecurity measures to prevent bacterial disease outbreaks in eel aquaculture.
**Keywords**: 16S rRNA, *Aeromonas*, aquaculture, bacterial pathogen, fish disease.

**Introduction**. Eel aquaculture in Indonesia possesses significant development potential, as eels (*Anguilla bicolor*) are high-value fishery commodities with rising demand in both domestic and international markets. Eels are highly sought after in countries such as Japan, Hong Kong, the Netherlands, Germany, Italy, and other export destinations, establishing this species as economically important for aquaculture development (Primyastanto, 2016). Nevertheless, eel farming in Indonesia is currently limited to several provinces, with East Java identified as a major eel-producing region. Despite considerable economic potential, the sector has not developed optimally because Indonesia continues to rely on wild-caught seed, particularly glass eels, due to the lack of established hatchery technology for large-scale seed production. This reliance presents a significant challenge for sustainable eel aquaculture and emphasizes the necessity of balancing production development with conservation efforts, especially by preventing overexploitation of glass eels for export.

Beyond seed availability, feed management constitutes a major constraint in eel farming, particularly during the rearing phase from glass eel to elver. The success of eel aquaculture is highly dependent on the implementation of appropriate grow-out technologies, especially during the transition from glass eel and juvenile stages to marketable size (Sudaryono et al., 2014). Banyumas Regency, Central Java, possesses substantial eel resources due to the presence of the Serayu River, which serves as a significant migration route for eels (Pratama et al., 2019). According to Saputra et al., (2021), eel aquaculture development in Banyumas has involved collaboration with the

Delta Sidat farmer group, recognized by the Banyumas Marine and Fisheries Office as technically and administratively qualified for eel culture. In practice, eels are intensively reared in fiber tanks utilizing phytoremediation and probiotic-based systems to maintain water quality and enhance fish growth. This technology has been shown to increase survival rates up to 89.87% and yield promising economic benefits for local eel farmers.

Despite technological advancements, bacterial diseases continue to represent a major constraint in eel aquaculture by adversely affecting fish health, survival, and production performance. The adaptation phase of wild-caught seed to captive culture conditions is particularly critical, as fish are highly susceptible to stress resulting from environmental changes. Multiple bacterial pathogens have been reported to infect eels, including *Aeromonas hydrophila* (Suryaningtyas & Sari, 2015; Alika et al., 2021; Nisa et al., 2025), *Pseudomonas luteola*, *Vibrio fluvialis*, *Aeromonas sobria*, and *Aeromonas caviae*, all of which may induce histopathological alterations in fish organs (Setyawan et al., 2015). These observations underscore the significance of bacterial infection as a health issue in eel aquaculture and highlight the necessity for accurate diagnostic methods.

Eels exhibit heightened vulnerability to disease due to their catadromous life cycle, which involves migration from freshwater habitats to marine environments for spawning (Linda et al., 2023). This biological trait renders eels highly sensitive to fluctuations in environmental parameters, particularly water quality and salinity. Sudden environmental changes can induce physiological stress and increase susceptibility to bacterial infections. In aquaculture systems, factors such as improper handling of wild-caught glass eels, inadequate water quality management, excessive stocking density, and prolonged stress can compromise fish health and result in mortality (Toro et al., 2024). Optimal water quality conditions for eel culture typically include temperatures of 26-30°C, pH values between 6.5 and 8.0, dissolved oxygen above 4 mg $L^{-1}$, ammonia below 0.02 mg $L^{-1}$, and salinity between 0 and 10 ppt. Maintaining these parameters supports metabolism and osmoregulation while minimizing chronic stress (Klau et al., 2020). Consequently, eel health management should employ an integrated approach, encompassing biosecurity, stocking density regulation, recirculating aquaculture systems, seed quarantine, water quality monitoring, and probiotic application to enhance fish immunity (Toro et al., 2024).

*Aeromonas* spp. are among the most frequently reported opportunistic bacterial pathogens in aquaculture, causing significant economic losses in freshwater and brackish water farming systems. Species such as *Aeromonas hydrophila*, *Aeromonas veronii*, and *Aeromonas dhakensis* are linked to motile aeromonad septicemia, ulceration, hemorrhage, and elevated mortality in various cultured fish species. The intensification of aquaculture, increased stocking densities, water quality fluctuations, and environmental stress are key factors contributing to the prevalence of *Aeromonas* infections in contemporary aquaculture systems (Rasmussen-Ivey et al., 2016; Fernández-Bravo & Figueras, 2020). Consequently, the detection and identification of *Aeromonas* spp. in cultured fish are critical for effective disease surveillance and the formulation of appropriate health management strategies.

The identification of bacterial pathogens is a critical step in fish disease diagnosis because it enables more accurate determination of infectious agents. Conventional identification methods based on colony morphology, Gram staining, and biochemical tests are useful for preliminary characterization but often have limited ability to differentiate bacteria with similar phenotypic characteristics. Consequently, molecular identification has been increasingly used because it relies on relatively stable genetic information and provides more specific results. This approach has been widely used in fish health studies to detect bacterial pathogens associated with cultured organisms, including *Aeromonas*, *Pseudomonas*, *Vibrio*, and *Edwardsiella*, which cause disease in fish (Noer, 2021). One of the most commonly used molecular markers for bacterial identification is the 16S ribosomal RNA gene. This gene encodes a component of the small ribosomal subunit in bacteria and is approximately 1,500 base pairs long. The 16S rRNA gene contains conserved regions that are relatively similar among bacterial groups and hypervariable regions that differ among taxa. These characteristics allow the use of universal primers for DNA amplification while providing sufficient sequence information for bacterial identification at the genus and, in some cases, species level. Therefore, the 16S rRNA gene has been widely used as a

molecular marker for bacterial identification, classification, and phylogenetic analysis (Akihary & Kolondam, 2020).

Polymerase Chain Reaction (PCR) is one of the most commonly used molecular methods for 16S rRNA gene analysis. PCR enables the amplification of target DNA fragments into millions of copies, allowing further analysis through gel electrophoresis, DNA sequencing, and sequence similarity comparison using genetic databases such as the Basic Local Alignment Search Tool (BLAST). Compared with conventional culture-based methods, molecular identification using PCR and 16S rRNA sequencing provides faster and more accurate identification of bacterial isolates. This approach has been widely applied in fish bacteriology. For example, Artati & Lubis, (2020) used PCR to detect *Aeromonas hydrophila*, a common bacterial pathogen in aquaculture. Zafrida et al. (2024) identified bacterial isolates from the intestine of selais fish (*Kryptopterus lais*) as *Proteus mirabilis* based on 16S rRNA gene analysis, while Hadiatun et al. (2025) identified several bacterial species, including *Pseudenterobacter timonensis*, *Ralstonia* sp., *Aeromonas salmonicida*, and *Stenotrophomonas maltophilia*, using PCR and 16S rRNA gene sequencing.

Despite the growing application of molecular identification techniques in aquaculture research, data on the diversity of bacterial pathogens associated with cultured eels in Indonesia remain scarce, particularly those utilizing molecular approaches. Molecular identification using 16S rRNA gene analysis is therefore essential to facilitate rapid, accurate, and evidence-based diagnosis of bacterial pathogens in eel aquaculture. In this context, the present study was conducted to detect and identify bacterial pathogens isolated from cultured eels in Notog Village, Patikraja District, Banyumas Regency, employing PCR amplification, 16S rRNA gene sequencing, BLAST analysis, and phylogenetic reconstruction.

## Material and Method

***Fish sampling***. The study was conducted in March-April 2026. Fish samples were obtained from an eel (*Anguilla bicolor*) farming site in Notog Village, Banyumas Regency, Central Java, Indonesia. The fish were collected from a single culture pond and placed in plastic containers for transportation to the laboratory. A total of 20 cultured eels were used as samples for bacterial examination. Liver tissues were selected for bacterial isolation because this organ is commonly associated with systemic bacterial infection and can reflect the health status of cultured fish. Eels were selected as the target species due to their high sensitivity to water-quality changes and their susceptibility to bacterial infection under suboptimal culture conditions.

***Bacterial culture***. The isolation of pathogenic bacteria from cultured eels was initiated by aseptic dissection using sterilized surgical instruments. Liver tissues were carefully collected as the primary source of bacterial isolates. The liver samples were homogenized to obtain a bacterial suspension, which was subsequently used as the inoculum for bacterial culture. The bacterial suspension was cultured on Tryptone Soya Agar (TSA) medium and incubated for approximately 24 h until bacterial colonies developed. Colonies grown on TSA were then subcultured onto Glutamate Starch Phenol Red (GSP) agar to support presumptive identification of bacterial isolates. The resulting colonies were observed macroscopically based on colony morphology, including size, shape, color, margin, elevation, and surface texture. Selected bacterial colonies were subsequently cultured in Tryptone Soya Broth (TSB) for genomic DNA extraction and further molecular analysis.

***DNA extraction***. Bacterial genomic DNA was extracted using the High Pure PCR Template Preparation Kit (Roche, Germany) according to the manufacturer's protocol with slight procedural adjustments. Briefly, 200 µL of bacterial suspension obtained from the TSB culture was transferred into a sterile microcentrifuge tube. The sample was then mixed with 200 µL of phosphate-buffered saline (PBS), 5 µL of lysozyme, 200 µL of Binding Buffer, and 40 µL of reconstituted Proteinase K. The mixture was homogenized thoroughly and incubated at 70°C for 10 min to ensure complete bacterial cell lysis. Following incubation, 10 µL of isopropanol was added to the lysate, and the mixture was gently mixed. The

mixture was subsequently transferred into a High Pure Filter Tube assembled with a Collection Tube and centrifuged at 8,000 × g for 1 min. The filtrate was discarded, and the Filter Tube was placed into a new Collection Tube. A total of 500 µL of Inhibitor Removal Buffer was then added to the upper reservoir of the Filter Tube, followed by centrifugation at 8,000 × g for 1 min. This washing step was repeated three times to remove potential PCR inhibitors and residual contaminants. After the final washing step, the Filter Tube–Collection Tube assembly was centrifuged at maximum speed for 10 s to remove any remaining Wash Buffer. The Collection Tube was then discarded, and the Filter Tube was transferred into a sterile 1.5 mL microcentrifuge tube. Genomic DNA was eluted by adding 200 µL of pre-warmed Elution Buffer at 70°C to the upper reservoir of the Filter Tube, followed by centrifugation at 8,000 × g for 1 min. The extracted DNA was stored at −20°C until further PCR amplification.

***PCR and sequencing***. Primers in this study used the universal primers 27F and 1492R. PCR amplification was performed following the Bioline protocol using MyTaq™ HS Red Mix (Suwarsito et al., 2026). PCR was performed in a total volume of 50 µL containing 2 µL of DNA template, 1 µL each of forward and reverse primers, 25 µL of MyTaq™ HS Red Mix, and 21 µL of nuclease-free water. Thermal cycling conditions consisted of an initial denaturation at 95°C for 1 minute, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 15 seconds, with a final extension at 72°C for 5 minutes. PCR products were visualized on 1.5% agarose gels using a 100 bp DNA ladder as a size marker. Successfully amplified products were sent to PT Genetika Sciences Indonesia for sequencing.

***Sequence and phylogenetic analysis***. The obtained bacterial DNA sequences were edited and aligned using the ClustalW algorithm implemented in MEGA 12 software. The aligned sequences were subsequently compared with reference sequences available in the GenBank database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) to determine the closest taxonomic affiliations of the bacterial isolates. Sequence similarity analysis was performed based on the percentage identity between the sample sequences and reference sequences retrieved from GenBank. Representative reference sequences with high similarity values were then used to reconstruct the phylogeny. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method in MEGA 12, and the reliability of the inferred tree topology was evaluated using 1,000 bootstrap replications.

## Results

***Bacterial isolation***. The morphological observation of bacterial colonies isolated from cultured eel in Notog Village revealed considerable variation in colony characteristics. The colonies ranged from small to large in size, with spindle-shaped to irregular forms, pulvinate elevation, entire to undulate margins, smooth surfaces, and red to yellow pigmentation. These phenotypic variations indicate that the bacterial isolates recovered from eel liver tissues may represent heterogeneous bacterial populations associated with the internal organs of cultured eels. Colony morphology remains an important preliminary approach in bacterial characterization because features such as shape, margin, elevation, surface texture, and pigmentation can provide initial information regarding bacterial diversity. However, colony morphology alone is insufficient for accurate identification, particularly for closely related aquatic bacteria that often share overlapping phenotypic traits.

***PCR amplification of 16S rRNA gene***. Amplification of the bacterial 16S rRNA gene successfully produced distinct DNA bands in both bacterial isolates (Sample 1 and Sample 2), as shown in Figure 1. Electrophoresis analysis revealed a single prominent band in each isolate, indicating successful amplification of the target fragment and the absence of significant non-specific products. Based on the migration pattern relative to the 100 bp DNA ladder, the amplified fragments were estimated to be approximately 1500 kb in

length, which corresponds to the expected size of the nearly full-length bacterial 16S rRNA gene. The presence of a single amplification product suggests that the extracted DNA possessed sufficient quality for downstream sequencing analysis. The successful amplification further confirms that the bacterial isolates recovered from cultured eel tissues were suitable for molecular identification using the 16S rRNA marker.

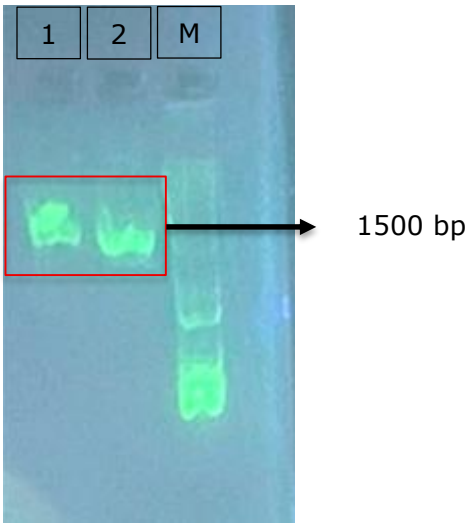


Figure 1. Amplification of the bacterial 16S rRNA gene (1 = sample code 1.4.3; 2 = sample code 4.4.3; M = marker 100 bp).

***Molecular identification based on BLAST analysis***. Sequence analysis of the amplified 16S rRNA gene fragments revealed that both isolates belonged to the genus *Aeromonas*. BLAST comparison against the GenBank database identified isolate 1.4.3 as *Aeromonas dhakensis* with a sequence similarity of 97.19%, whereas isolate 4.4.3 showed the highest similarity to *Aeromonas veronii* at 96.86% (Table 1). The obtained similarity values indicate a close phylogenetic relationship between the isolates and their respective reference strains. According to commonly accepted bacterial taxonomic thresholds, 16S rRNA sequence similarities above 97% generally indicate affiliation within the same genus, whereas species-level identification often requires similarity values exceeding 98.7-99.0% together with additional molecular markers. Conventional taxonomic approaches that stop at the species level have proven inadequate for disease control, thereby necessitating the adoption of high-resolution methods such as whole-genome sequencing (WGS), multilocus sequence typing (MLST), and Capsular Polysaccharide CPS to map clonal variation, virulence, and AMR (Pratama et al., 2026).

Table 1

Sequence analysis of BLAST comparison against the GenBank database

| *Sample code* | *Search result of similarity* | *Percentage (%)* |
|---|---|---|
| 1.4.3 | *Aeromonas dhakensis* | 97.19 |
| 4.4.3 | *Aeromonas veronii* | 96.86 |

***Phylogenetic relationships of the bacterial isolates***. Phylogenetic reconstruction based on 16S rRNA sequences demonstrated that both isolates clustered within the *Aeromonas* clade and were clearly separated from the outgroup species *Streptococcus agalactiae*. The topology of the phylogenetic tree revealed a close relationship between the isolates and several pathogenic *Aeromonas* species, including *A. aquariorum*, *A. taiwanensis*, *A. dhakensis*, *A. caviae*, *A. rivipollensis*, *A. veronii*, and *A. hydrophila*, as shown in Figure 2. Bootstrap values supporting the major nodes ranged from moderate to high, indicating acceptable confidence in the inferred phylogenetic relationships. The clustering pattern confirms the BLAST results and suggests that the isolates recovered from cultured eel were evolutionarily associated with pathogenic members of the genus *Aeromonas*.

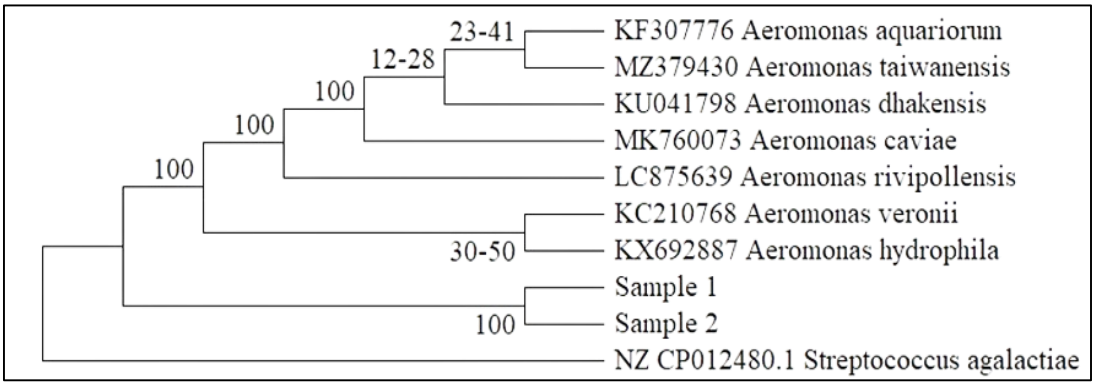

Figure 2. Phylogenetic reconstruction based on 16S rRNA sequences.

**Discussion**. The observed colony characteristics showed partial similarity to those commonly reported for members of the genus *Aeromonas*. *A. hydrophila*, for example, is a Gram-negative, motile, non-spore-forming, non-capsulated, facultatively anaerobic bacterium that is generally oxidase- and catalase-positive. This species is also known to ferment glucose and maltose, producing acid, and to reduce nitrate. On general agar media, *A. hydrophila* may produce circular colonies with smooth margins, a convex surface, a shiny appearance, and a translucent appearance after approximately 24 h of incubation (Lenchenko et al., 2022). Nevertheless, the phenotypic similarity among *Aeromonas* species makes conventional identification challenging. Therefore, the colony characteristics observed in the present study should be interpreted as preliminary evidence supporting the presence of aeromonad-like bacteria rather than as definitive species-level identification.

Colony morphology is influenced by bacterial species, culture medium, incubation conditions, and physiological state of the cells. In this study, spindle-shaped and irregular colonies were observed, whereas bacterial colonies in general may also appear circular, filamentous, or rhizoid. The pulvinate elevation observed in the isolates indicates a raised, cushion-like colony structure, which may reflect intensive bacterial growth on the culture medium. Similar colony elevation patterns have been reported in culturable bacterial isolates from environmental samples, including those from the Khewra salt mine in Pakistan (Akhtar et al., 2008). The colony margins also varied, with some isolates showing entire margins and others displaying undulate margins. Entire margins generally indicate more uniform radial colony expansion, whereas undulate margins may reflect uneven growth patterns or differences in colony development (Sousa et al., 2015). Pigmentation was another notable characteristic of the bacterial colonies recovered in this study. The red and yellow coloration observed in several colonies may be associated with the production of bacterial pigments. Red pigmentation in bacteria is commonly linked to prodigiosin-like compounds, whereas yellow pigmentation is often associated with carotenoid pigments. These pigments may provide ecological advantages for bacteria, including protection against oxidative stress and adaptation to environmental fluctuations (Choi et al., 2021). However, pigment-based interpretation should be approached with caution because colony color can be influenced by culture medium composition, incubation time, temperature, and nutrient availability. Therefore, pigmentation is useful for descriptive characterization but should not be used as a primary criterion for taxonomic assignment.

Molecular identification based on the 16S rRNA gene provided stronger evidence that the bacterial isolates belonged to the genus *Aeromonas*. Amplification using universal bacterial primers successfully produced clear DNA bands of approximately 1500 bp, corresponding to the expected size of the nearly full-length bacterial 16S rRNA gene. The presence of a single prominent amplification product indicated that the extracted bacterial DNA was suitable for sequencing and subsequent molecular analysis. The use of 16S rRNA sequencing is widely accepted for bacterial identification because this gene contains conserved regions suitable for universal primer binding and variable regions that enable taxonomic discrimination among bacterial groups (Singh et al., 2012). BLAST analysis revealed that isolate 1.4.3 showed the highest sequence similarity to *A. dhakensis* at 97.19%, whereas isolate 4.4.3 showed the highest similarity to *A. veronii* at 96.86%. These

results indicate that both isolates are closely related to members of the genus *Aeromonas*. However, the similarity values obtained in this study should be interpreted carefully. Although 16S rRNA sequence similarity of 97% or higher may support genus-level affiliation, definitive species-level identification in bacteria generally requires a higher similarity, typically 98.7-99.0%, together with additional genetic evidence. Therefore, the isolates in this study can be confidently assigned to the genus *Aeromonas*, while their identification as *A. dhakensis* and *A. veronii* should be considered putative until confirmed using higher-resolution molecular markers.

The phylogenetic analysis further supported the BLAST results by placing both isolates within the *Aeromonas* clade and separating them from the outgroup species *S. agalactiae*. The clustering of the isolates with pathogenic *Aeromonas* species, including *A. dhakensis*, *A. veronii*, *A. hydrophila*, *A. caviae*, *A. aquariorum*, and related taxa, confirms their evolutionary relationship with bacteria frequently associated with fish disease. This phylogenetic position is important because members of the genus *Aeromonas* are widely recognized as opportunistic pathogens in freshwater aquaculture systems. These bacteria are ubiquitous in aquatic environments and can become pathogenic under stressful culture conditions, including poor water quality, high stocking density, elevated organic matter, and handling stress (Fernández-Bravo & Figueras, 2020).

The detection of *Aeromonas* spp. in the liver tissues of cultured eels is particularly important because the liver is an internal organ involved in systemic bacterial infection. The presence of aeromonads in this organ suggests that the bacteria were not merely external contaminants but may have colonized internal tissues. In fish, *Aeromonas* infections are frequently associated with motile aeromonad septicemia, hemorrhagic lesions, ulceration, fin erosion, exophthalmia, and systemic disease (Semwal et al., 2023). Such infections can result in reduced growth performance, increased mortality, and significant economic losses in intensive aquaculture systems (Rasmussen-Ivey et al., 2016). Therefore, the detection of *Aeromonas* spp. in cultured eels from Banyumas indicates a potential bacterial health risk that should receive attention in eel farming management.

Among the detected isolates, the putative identification of *A. dhakensis* is noteworthy, as this species is increasingly recognized as an emerging pathogen in aquatic animals. Historically, *A. dhakensis* was often misidentified as *A. hydrophila* due to their phenotypic similarity and the limited resolution of conventional biochemical methods. Molecular studies have shown that *A. dhakensis* may harbor several virulence-associated genes, including those encoding hemolysins, enterotoxins, proteases, and other extracellular enzymes involved in tissue damage and host invasion (Janda & Abbott, 2010). Its detection in cultured eel suggests that this bacterium may contribute to the risk of bacterial disease in eel aquaculture systems in Banyumas. Continuous monitoring is therefore required to determine its prevalence, pathogenicity, and possible association with clinical disease in farmed eel populations. The second isolate showed the closest relationship to *A. veronii*, another important bacterial pathogen in freshwater aquaculture. *A. veronii* has been reported in several farmed fish species, including tilapia (Fadel et al., 2025), carp, catfish (Adah et al., 2024), and eel (Korun et al., 2019), and is frequently associated with septicemic disease and mortality events. This species possesses multiple virulence determinants that facilitate host colonization, tissue invasion, immune evasion, and disease progression (Liu et al., 2022). The detection of *A. veronii*-like bacteria in cultured eels may therefore have important implications for fish health, especially in intensive systems where environmental stressors can increase host susceptibility to infection.

Environmental conditions play a critical role in the emergence of *Aeromonas*-associated disease. Poor water quality, excessive organic loading, fluctuating temperatures, suboptimal dissolved oxygen, and high stocking density can increase bacterial proliferation while weakening fish immune responses (Mulia et al., 2025). In eel aquaculture, these risks may be amplified by eels' physiological sensitivity to environmental change and their dependence on stable water quality during culture. Consequently, the occurrence of *Aeromonas* spp. in the present study may also indicate the need to strengthen environmental monitoring and biosecurity practices at the farm level. Although 16S rRNA sequencing is useful for bacterial identification, this marker has

limitations in resolving closely related species within the genus *Aeromonas*. Several *Aeromonas* species share highly conserved 16S rRNA sequences, which may result in overlapping similarity values and ambiguous species assignments. Previous studies have demonstrated that sequence variation among *Aeromonas* strains may be relatively low, and additional genes are often required for reliable species discrimination (Yáñez et al., 2003). Therefore, future studies should incorporate housekeeping genes such as gyrB, rpoD, or recA, or apply multilocus sequence analysis (MLSA) to improve taxonomic resolution (Beaz-Hidalgo & Figueras, 2013). The inclusion of virulence gene detection and antimicrobial susceptibility testing would also provide more comprehensive information regarding the pathogenic potential and management risks of the isolates.

In summary, this study demonstrates that molecular analysis targeting the 16S rRNA gene is effective for detecting pathogenic bacterial groups associated with cultured eel. The identification of isolates within the genus *Aeromonas* underscores the necessity of routine bacterial surveillance in eel aquaculture systems. Fish can withstand infections, but when environmental conditions deteriorate or stress occurs, their immune systems weaken, making it easier for pathogens to infect and cause death (Pratama et al., 2022). Preventive health management should encompass regular water quality monitoring, reduction of excessive stocking densities, enhancement of sanitation and biosecurity, quarantine of newly introduced seed, and early molecular diagnosis of bacterial pathogens. These strategies are vital for minimizing the risk of bacterial disease outbreaks and supporting sustainable eel aquaculture in Banyumas.

**Conclusions**. The present study successfully detected and identified pathogenic bacterial isolates from cultured eels in Notog Village, Patikraja District, Banyumas Regency using 16S rRNA gene-based molecular analysis. PCR amplification using universal primers 27F and 1492R produced clear DNA bands of approximately 1500 bp, indicating successful amplification of the bacterial 16S rRNA target region. BLAST analysis revealed that the two bacterial isolates were closely related to members of the genus *Aeromonas*, with isolate 1.4.3 showing the highest similarity to *Aeromonas dhakensis* and isolate 4.4.3 to *Aeromonas veronii*. Phylogenetic reconstruction further supported these findings by placing both isolates within the *Aeromonas* clade and separating them from the outgroup species *Streptococcus agalactiae*.

The detection of *Aeromonas* spp. in liver tissues of cultured eel suggests the presence of opportunistic bacterial pathogens within the aquaculture environment. These bacteria can pose significant health risks, especially under stressful culture conditions such as poor water quality, high stocking density, and insufficient biosecurity. While 16S rRNA sequencing effectively assigned the isolates to the genus *Aeromonas*, the similarity values obtained indicate that definitive species-level identification requires further validation using higher-resolution molecular markers. Accordingly, routine molecular monitoring, enhanced water quality management, and stringent biosecurity measures are recommended to support disease prevention and promote sustainable eel aquaculture in Banyumas.

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**Authors Contributions**. IP conceived and designed the study, prepared the original draft of the manuscript, and conducted the molecular identification; MNH and BBG contributed to the preparation of the original manuscript draft and, together with DJY, ISJ, and AM, participated in data collection and bacterial isolation; AFA and IST assisted IP in conducting the molecular identification. All authors reviewed and edited the manuscript and approved the final version for publication.

**Conflicts of Interest**. The authors declare that there is no conflict of interest.

**Data Availability**. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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