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Rapid Detection of the *Aeromonas* sp. group using Conventional PCR at the Aquaculture Technology Development Center, Cangkringan, Sleman, Special Region of Yogyakarta

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Abstract

The catfish is a freshwater cultivated fish that can be found all over Indonesia. However, the problems that occur in general are usually attacks by pathogens from the *Aeromonas* sp group. This research aims to optimize the rapid detection of the *Aeromonas* sp group using PCR at the Aquaculture Technology Development Center, Cangkringan, Sleman, special region of Yogyakarta. The research was conducted by survey with purposive sampling. Isolation was carried out using two growth media, namely Tryptone Soy Agar (TSA) and Glutamate Starch Phenile (GSP). Colonies that had characteristics of the *Aeromonas* sp group were subjected to PCR amplification using specific primers for the *Aeromonas* sp group. The results of the amplification resulted in DNA that matched the target, namely 953bp, which showed that the sample belonged to the *Aeromonas* sp group. Based on this technique, the level of accuracy in detecting pathogens is higher than conventional methods.

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Introduction

Cultivation productivity in the Yogyakarta Special Region shows varying production values in the last four years measured from 2017 to 2020. According to statistical data on aquaculture production, catfish production in the Special Region of Yogyakarta in 2017 reached 52,024 tons and in 2020 it reached 40,826 tons (Central Statistics Agency). The decline in catfish production that occurred was quite large and one of the reasons was due to attacks by pathogenic bacteria. This is a serious problem for cultivators so it needs early treatment at the start of cultivation. As a government agency, the Cangkringan Aquaculture Technology Development Center (BPTPB), Sleman Special Region of Yogyakarta is trying to follow up to study and provide solutions related to problems occurring in the field.

Aeromonas sp. is one of the Gram-negative pathogenic bacteria that commonly attacks farmed fish which can result in large losses of up to 100%. *Aeromonas* sp. known as an opportunistic pathogen in fish. However, under certain conditions such as stress and decreased immune function it can become a major pathogen in fish (Kundan et al., 2015). *Aeromonas hydrophila* strains cause Motile Aeromonas Septicemia (MAS), which is caused by strains of this group (Zhang et al., 2020). *A. hydrophila* attacks are characterized by reddish to hemorrhagic symptoms on the surface of the fish's body. It was discovered that *A. hydrophila* attacks caused severe damage to internal organs, including the spleen, in addition to surface damage (Baumgartner et al., 2017). *Aeromonas sp* group cause disease in fish, namely *A.janaei, A. veroni*i in Nila Fish (Dong et al. 2017) *A. caviae, A. sobria* and *A. hydrophila* were found to attack catfish (Anyanwu et al., 2015).

Selection of pathogenic bacteria Aeromonas sp. can be done using specific media to group at the genera level. *Aeromonas sp* group can grow on Glutamate starch phenyl (GSP) medium with a certain incubation period so that it can be used for initial selection of this group (Gavriel & Lamb 1995; Lee & Wendy 2017). However, current technological developments require some cultivation supervisors to detect early the presence of pathogenic bacteria. Polymerase Chain Reaction (PCR) is a tool for duplicating up to billions of target DNA sequences in a short time. This technology can be an alternative in detecting pathogens based on target genes using specific primers. In addition, this method is considered more accurate than conventional methods. This research aims to optimize the rapid detection of the Aeromonas sp group. using polymerase chain reaction at the Cangkringan aquaculture technology development center in the special region of Yogyakarta.

Materials and methods

The research was carried out using a survey method where samples of Aeromonas sp bacteria. taken from catfish and cultivation water in the Wonocatur area of Yogyakarta. This research uses a purposive sampling technique. Selected fish samples are taken based on the disease symptoms they cause. This research was carried out from February to March 2023 at the disease laboratory of the Aquaculture Technology Development Center (BPTPB) Cangkringan Special Region of Yogyakarta.

Bacterial Sampling

Bacterial samples were taken using the streak plate method on Tryptone Soy Agar (TSA) and Glutamate Starch Phenile (GSP) media then incubated for 24 hours at 28°C. The growing colonies were isolated based on the general colony morphology of *Aeromonas* sp. Then purification was carried out using inclined tube media. The use of GSP medium aims to obtain specific *Aeromonas* sp. indicated by a change in media color from red to yellow (Lee and Wendy 2017).

PCR Amplification

The initial stage carried out is DNA extraction. This step was carried out using a Genomic DNA Kit with procedures according to the recommendations of the modified kit (Transgen Biotech) https://www.transgenbiotech.com/data/upload/pdf/EE101_2023-03-30.pdf. Bacteria were cultured on TSA and GSP media then the bacterial isolates were directly diluted in 200 µL sterile water using a loop needle. The DNA extraction process generally has three stages, namely DNA lysis, DNA binding, and DNA washing so that pure DNA is obtained which can be used as a template for PCR amplification.

The next stage, namely amplification, is carried out using a PCR Thermocycler. Two pairs of primers were used in this study, namely a pair of primers Aero-16s_F (5'-CTACTTTTGCCGGCGAGCGG-3') and Aero-16s_R (5' TGATCCCGAAGGCACTCCC-3') with amplification results measuring 953bp for the detection of *Aeromonas* sp. generally at the genus level (Nhinh et al. 2021). The PCR mastermix contains a pair of primers, nuclease free water and 2×EasyTaq® PCR SuperMix (DNA polymerase, Buffer MgCl2, and dNTP) with respective volumes according to the protocol https://www.transgenbiotech.com/data/upload/pdf/AS111_2023-03-30.pdf. The PCR program used for amplification was predenaturation at 94ºC for 2 minutes; followed by 35 cycles: denaturation at 94ºC for 20 seconds, annealing at 55ºC for 30 seconds and extension at 72ºC for 20 seconds and final extension at 72ºC for 5 minutes and final temperature 25ºC for 1 minute. The PCR results were seen using gel electrophoresis visualization with a band length of 953bp.

Data Analysis

The data in this study are four representative bacterial isolates from several bacteria that show typical colony characteristics on GSP and TSA media. PCR

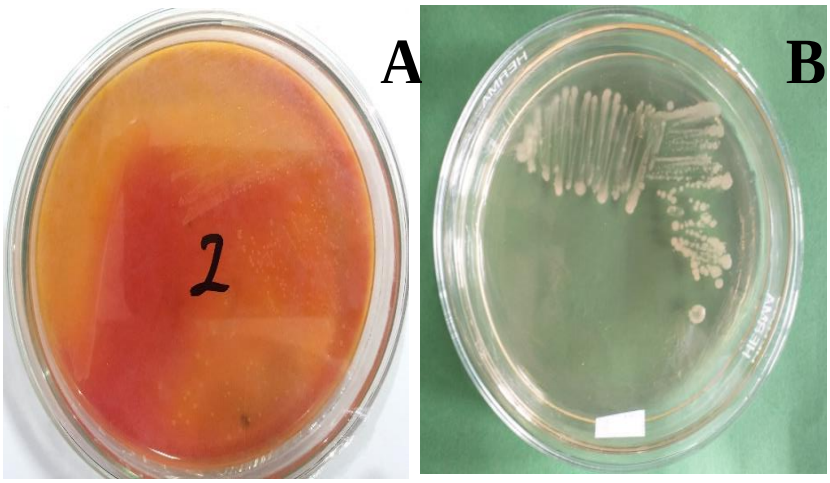

Figure 1. Results of Isolation of *Aeromonas* Bacteria; (A): GSP media; (B) TSA media

amplification results were visualized on agarose gel electrophoresis. The data is analyzed descriptively, displayed in the form of images and then discussed in a complex manner based on scientific studies and references.

Results

The pathogen isolate *Aeromonas* sp. successfully isolated from sick catfish and rearing media in one area in Yogyakarta. The isolate showed a characteristic yellow color on GSP media which had been incubated for 24 hours at 28ºC. Image of *Aeromonas* sp isolate. shown in figure 1 and table 1.

Amplification of the isolate that grew and produced a yellow color on GSP media was then performed using specific primers for *Aeromonas* sp. The results of PCR amplification are shown in Figure 2.

Based on the PCR amplification results, it showed success in obtaining targeted DNA with a length of around 953bp. This was shown in all samples, indicating that the test samples were bacteria from the *Aeromonas* sp group. to ensure that

Table 1. Morphology of the bacterial colony

Code isolate	Colony color on GSP media
LT01	Yellow
AT01	Yellow
LG01	Yellow
AG01	Yellow

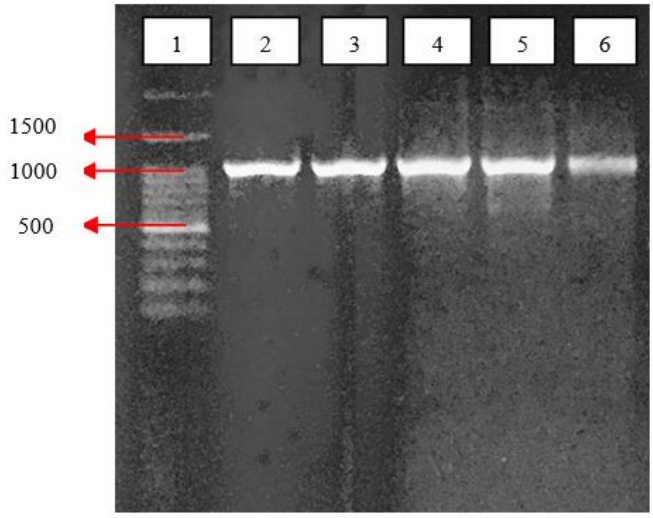

Figure 2. PCR amplification of the *Aeromonas* sp group. No 1. DNA marker, 2. Positive control, 3. LT01, 4. AT01, 5. LG01, 6. AG01

the sample belongs to the *Aeromonas* sp group. we have added *Aeromonas hydrophila* as a positive control and showed the same amplification results.

Discussion

Aquaculture is a sector of the fishing industry that makes a significant contribution to food security. Various problems often arise in the process of cultivating fish, including catfish. Even though catfish have quite good resistance to attacks by pathogens, under certain conditions catfish that are exposed to pathogenic bacteria can experience damage to organs and tissues and even lead to mass death. Research by Pramudita et al (2013) shows that *Aeromonas salmonicida* and *Aeromonas hydrophila* attacks on catfish cause clinical symptoms in several parts of the body and result in deaths of 35% and 20%. In this study we attempt to address problems in fish farming. The initial step that we took as an effort to deal with this problem was to quickly detect it using the PCR method, especially targeting the pathogen Aeromonas sp. which is a common pathogen in waters (Ekawati et al., 2017; Andriyanto et al., 2020; Setiadi & Wadjdy, 2021).

A conventional detection method uses specific media, such as GSP media that is specifically designed for growing *Pseudomonas* sp and *Aeromonas* sp, these indicators change color on the media as the organism grows (Pusparani *et al.*, 2021). However, this method requires confirmation of its accuracy in detecting target bacteria. Molecular-based detection is now available

to detect rapidly on specific media while confirming detection results. We found very interesting research results where the *Aeromonas* sp. can be detected using specific primers at the genus level (Nhinh *et al.,* 2021). Apart from this research, detection of the pathogen group Vibrio sp. has also been carried out as identification for monitoring cultivation of vannamei shrimp (Han *et al.,* 2019). These two studies inspired it to be developed as a method for carrying out routine monitoring in the Yogyakarta area.

Aeromonas sp group is one of the common bacteria in freshwater environments. *Aeromonas* sp is a pathogen that attacks all types of cultivated fish commodities, including catfish. The *Aeromonas* sp group has opportunistic pathogenic properties, meaning that it can infect fish under certain conditions, such as unhealthy fish conditions, polluted waters, and an abundance of cells. *Aeromonas* sp can cause histological changes, total hemocytes, and necrotic cells in the gills (Mulia et al., 2023). Several Aeromonas were found to infect catfish with high virulence including *Aeromonas* spp., *A. hydrophila, A. caviae, A veronii* bv veronii, and *A. dhakensis* (Mulia et al., 2023). The characteristics of *Aeromonas* sp can be determined based on biochemical tests. The characteristics of *Aeromonas* sp are reported to include gram negative, positive motility, catalase positive, oxidase positive (El-Sharaby et al., 2021), fermentative (Anwar and Tugiyono, 2023), indole positive and has the ability to ferment glucose, inositol and adonitol as carbon source without producing gas (Dwi et al., 2023). Identification of *Aeromonas* sp via GSP media is aimed at the presence of a yellow zone on the media (Pusparani et al., 2021).

Conclusion

According to the research conducted, conventional PCR has higher accuracy in identifying *Aeromonas* sp than conventional based on biochamical in identifying *Aeromonas* sp. The amplification results showed that DNA matched the target, namely 953bp, which showed that the sample belonged to the *Aeromonas* sp group.

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