JURNAL VOKASI INDONESIA
Journal of Vocational Program University of Indonesia

6/1 (2018), 47-50

# *The Isolation DNA* Chromosomal DNA from Local Isolate of *Aeromonas hydrophila* Bacteria from East Java

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Diterima : 15 Agustus 2017
Layak Terbit : 19 Desember 2017

## Abstract

Aeromoniasis is a bacterial disease caused by *Aeromonas hydrophila* that affects fish and shrimps in ponds and aquariums. This is an ulcer disease that causes petechiae in scales of fish and may be fatal. It can cause economic loss if it is not treated with medication accompanied with improved sanitation. It affects numerous freshwater fish farms in East Java. A previous study has characterized an antigenic protein derived from the outer membrane protein of *A. hydrophila*. In order to perform sequencing of the DNA coding for this protein, we first conducted a study for isolating DNA fragments of *A. hydrophila* via four stages: cultivation and harvesting of bacterial cells, cell lysis, DNA purification, and the concentration of chromosomal DNA. The results will be used as a predictive immunogenic determinant by the method by Kolaskar and Tongaonkar.

**Keywords:** *Aeromonas hydrophila*, chromosomal DNA, immunogenic determinant

## *Abstraksi*

***Kromosom DNA Isolasi Bakteri Aeromonas Hydrophilla Isolate Lokal Jawa Timur. Penyakit Aeromonasis atau disebut Penyakit Bisul yang disebabkan oleh Aeromonas hydrophila.*** *Penyakit ini sering menyerang ikan dan udang di kolam atau akuarium. aeromonasis menunjukkan gejala klinis petechiae dalam skala dan menyebabkan kematian. Aeromonasis dapat menyebabkan kerugian ekonomi jika tidak diobati dengan pengobatan yang disertai dengan perbaikan sanitasi. Penyakit ini banyak menyerang peternakan ikan air tawar di Jawa Timur. Dalam penelitian sebelumnya telah berhasil mengkarakterisasi protein antigenik yang berasal dari OMP (protein membran luar) Aeromonas hydrophilla, oleh karena itu perlu dilakukan sequencing protein-coding DNA. Untuk mencapai tujuan tersebut kemudian dilakukan beberapa metode penelitian laboratorium eksploratif yaitu: Isolasi fragmen DNA Aeromonas hydrophilla melalui empat tahap, budidaya sel dan pemanenan sel bakteri, lisis sel, DNA Purification and Concentration chromosomal DNA. Hasilnya akan dijadikan prediktor imunogenik prediktif dengan metode Kolaskar dan Tongaonkar.*

***Kata Kunci****: Aeromonas hydrophilla, DNA kromosom, determinogenik determinan*

## INTRODUCTION

The genus *Aeromonas* has been recognized as an important pathogenic agent in freshwater fish farms for more than a decade. Infections caused by *Aeromonas hydrophila* and *Aeromonas sobria* have caused severe population loss in cultivated fish. Manifestations of *Aeromonas* infection include hemorrhagic septicemia, basal ulceration, symptom septicemia, exophthalmoses, and numerous other signs.

Control of the disease by antibiotics may not always be successful, and the use of antibiotics can be harmful to the environment because resistant bacteria can be selected and resistance can be transferred to fish pathogens or even human pathogens and other harmful bacteria. Therefore, the development of a vaccine is essential for protecting fish against the bacteria. However, high antigenic diversity among *A. hydrophila* isolates poses a major problem. Some virulence factors may contribute to the total virulence of these bacteria, including extracellular products, the S-layer, and adhesins. The structure of *A. hydrophila* is unlike

that of other gram-negative bacteria. *A. hydrophila* has villi and flagella on its outer surface comprising two components that have been identified as potential virulence factors: outer membrane proteins and lipopolysaccharides.

The outer membrane proteins of gram-negative bacteria are potential antigens that can directly induce specific hormonal immune responses mediated by β-cell lymphocytes, resulting in the rapid formation of antibodies. This protein has a role to show immunogenic characteristic in a particular molecular weight (Jawetz et al. 2004).

The nature and character of the local isolate of *A. hydrophila* from East Java differs from that of isolates from other regions. Environmental influences, such as climate, weather, and temperature can cause differences among strains of bacteria. This research aimed to find and characterize the protein-coding genes of the local isolate of *A. hydrophila*. The sequence of the protein-coding genes of the local isolate of *A. hydrophila* will be compared with sequencing data from GenBank for the determination of their homologies with strains from other regions. Information on homologies and differences in the location of nucleotides can be used to create the ingredients of a subunit vaccine. To accomplish this objective, the first step will be the isolation of the chromosomal DNA of the local isolate of *A. hydrophila*.

Based on the research background above, it can be formulated with the following problems: how does the DNA result of degradation protein of *A. hydrophila* bacteria isolate local East Java and how much does the DNA protein content of *A. hydrophila* isolate local East Java?

## MATERIALS AND METHODS

### Cultivation and harvest of *A. hydrophila* bacterial cells

The isolate of *A. hydrophila* was obtained from several fish and freshwater ponds in East Java: from goldfish and freshwater ponds in the Batu region, from gourami and freshwater ponds in Tulungagung, and from *Tilapia* and freshwater ponds in Sidoarjo. Fish that were suspected to be infected by ulcer disease or red sore disease were collected and transferred to the microbiology laboratory of the School of Veterinary Medicine of the Universitas Airlangga Surabaya for diagnosis. The isolates were cultured in tryptic soy agar (TSA) using biochemical test.

### Lysis of *A. hydrophila* bacterial cells

Cell degradation was performed using sonication. *A. hydrophila* was washed with phosphate-buffered saline (PBS) and centrifuged at 8000 rpm for 15 minutes. The *A. hydrophila* pellet was dissolved in 1 ml of PBS and sonicated at 20 Hz for 4 × 4 minutes at intervals of 2 minutes. The supernatant was taken and centrifuged again at 8000 rpm for 15 minutes. The supernatant was then stored for the analysis of proteins. Protease inhibitors were added for the prevention of protein damage.

### Purification of chromosomal DNA

The *A. hydrophila* sample was suspended in PBS, the suspension was transferred into an Eppendorf tube with a micropipette, to which 1000 µl of DNAzol solution was added. After being mixed well, the suspension mixture was incubated at room temperature for 2–15 minutes. The mixture was then centrifuged at 10,000 rpm for 10 minutes at 40°C. Next, 500 µl of the supernatant was transferred into an Eppendorf tube, and 500 µl of absolute ethanol was added. The DNA appeared to hover, and after centrifuging at 10,000 rpm for 10 minutes at 40°C, the DNA was precipitated. In the last step, the DNA was washed by adding 1000 µl of 75% ethanol and centrifuging again at 10,000 rpm for 10 minutes at 40°C. The DNA was carefully dried for removing the supernatant entirely, and the tube was left open for 5 minutes. The DNA was then dissolved in 50 µl of NaOH (pH 7.5) and stored at 40°C (Suwarno 2010).

### Determination of DNA concentration

DNA concentration was determined using UV spectrophotometry at a wavelength of 260 nm, where an absorbance value of 1.0 is equivalent to 50 µg of double-stranded DNA/ml. The purity of DNA can also be determined by the ratio. A purity ratio less than 1.8 indicates that the DNA is not sufficiently pure.

## RESULTS AND DISCUSSION

### Cultivation and harvest of *A. hydrophila* bacterial cells

The research sample of *A. hydrophila* local East Java isolate was obtained from fish infected by aeromoniasis in the Sidoarjo Blitar and Malang regions. The sampled fish (gouramis, catfish, and goldfish) are the largest fish commodities in these regions. *A. hydrophila* local isolates were propagated (Figure 5.1) by culturing on TSA medium and were incubated for 18–24 hours (Figure 5.2).

Cell degradation was performed using sonication. *A. hydrophila* from solid media was washed with PBS and centrifuged at 8000 rpm for

15 minutes (Figure 5.3). The *A. hydrophila* pellet was dissolved in 1 ml of PBS and sonicated at 20 Hz for 4 × 4 minutes at intervals of 2 minutes (Figure 5.4).

**Purification of chromosomal DNA**

After lysis of the bacterial cell walls, DNA molecules were separated from RNA and protein contaminants as well as from cell debris.

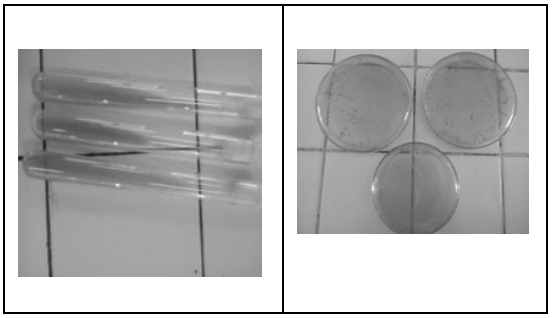

**Figure 1. *Hydrophila* Isolate In Stock Medium And In TSA Medium**

The standard method of agarose gel electrophoresis was used for identification, separation, and purification of DNA fragments.

Electrophoretic migration of DNA through the agarose gel was influenced by the size and conformation of the DNA molecule, the concentration of agarose, the electric current, and the temperature. The results of agarose gel electrophoresis of chromosomal DNA of *A. hydrophila* are shown in Figure 5.5.

**Marker DNA**

The length of the DNA of the *A. hydrophila* degradation protein was 400 bp.

**Figure 2 Washing of *A. hydrophila* isolate by centrifugation Degradation of cell walls of *A. hydrophila* using sonication**

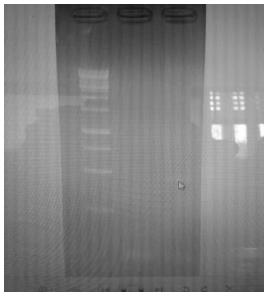

**Figure 3. Results of agarose gel electrophoresis of chromosomal DNA of *A. hydrophila***

The DNA was then assessed for its purity and quantified using a UV spectrophotometer.

**Determination of DNA concentration**

Quantitative DNA test was performed using UV-Vis spectrophotometer which involves nanotechnology; the pure DNA can absorb UV light via their purine and pyrimidine bases. Double-stranded DNA absorbs UV light at 260 nm. In the present study, a DNA concentration of 51.4 ng/µl was obtained.

## ACKNOWLEDGEMENTS

DNA that has been isolated can be used for the PCR test, by performing Sequencing Fragment Gen OMP A. Hydrophilic isolate East Java and continued with homology analysis and phylogenetic analysis. The result will be used as predictive determinant immunogenic.

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