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Bacterial Plasmids Profile from *Escherichia coli* Resistant to Metronidazole and Nalidixic Acid

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Abstract

Escherichia coli have a potency to be pathogen bacteria that cause an illness. Antibiotic treatments to a patient have a purpose to eliminate the pathogen bacteria. Bacteria resistance to antibiotic was influenced by the intensity of antibiotic treatment in a region, the uncontrolled antibiotics treatments would increase the antibiotic resistance of bacteria. Plasmids was an extrachromosomal DNA that encodes a functional protein that would eliminate the antibiotic activity. Plasmid is the determinant of bacteria sensitivity to antibiotics. In this case it would be important to find out the bacterial plasmid profile on the E.coli resistant to metronidazole and nalidixic acid antibiotics. This research was using four different sample from faces of diarrhea, ice block, waters from well, and ketchup to cultivate the E. coli. lasmid isolation method was carried out by lyses alkali method. Plasmid profile of the E. coli that resistant to metronidazole and nalidixic acid antibiotics and analyzed using electrophoresis on 1% agarose. E. coli plasmid DNA profile was observed as a fluorescent DNA band in ultraviolet rays. In result, isolated plasmids from bacteria that resistant to antibiotics metronidazole and nalidixic acid having similar size approximately 500 bp, different from bacteria that sensitive to antibiotics metronidazole and nalidixic acid has a smaller size in region of 100 bp.

1. Introduction

Escherichia coli is a member of the Enterobacteriaceae family, rod-shaped, Gram negative in a normal condition E. coli live in the intestine, it is also one among the facultative anaerobic bacteria. More than 700 serotypes of E. coli have been found. Serotypes classifying of E. coli based on O, H, and K

antigens. E. coli strain from pathogenic diarrheagenic (microorganisms that cause diarrhea) are classified by the virulence factors characteristic that only belongs to this bacteria. Therefore, the first analysis of pathogenic E. coli is usually required before testing for virulence marker (Todar 2008).

Antibiotics are a group of compounds that work by inhibiting the growth of bacteria (bacteriostatic) or cause the death of bacteria (bactericidal). This antibiotic worked through 5 main mechanisms that inhibit: 1). replication process, 2). transcription process, 3). translation process, 4). peptidoglycan synthesis, 5). tetrahydrofolate acid synthesis (Lamont 2006).

The main mechanism of the microbial population to survive is by genetic mutations, expression of a latent resistance gene, or through gene that has resistance determinant. The three mechanisms can be found together in a bacterium. Excessive use of antibiotics can lead to the selective pressures that drive the proliferation of resistant microorganisms (Yenny & Herwana, 2007).

Antibiotics dose that are less precise and careless consumption will raise the bacteria to resistant state. There are three types of resistance, they were non-genetic resistance, genetic resistance, and cross-resistance (Wibowo et al. 2011).

Resistance is the reduction of the influence of anti-infective drugs against bacteria. Another understanding of resistance is a failure of treatment by an antibiotic with therapeutic doses (Widodo, 2011). The resistance through mutation process was still not known because some species of microorganisms are mutating spontaneously. The plasmids resistence can transfer not only in one species but also between species in single genus. Resistance mechanism associated with membrane permeability (including outer membrane) which is encoded by chromosomally(Rahayu, 2006)

In general, their existence is separated from the main chromosome and replicate independently on the main bacterial chromosome, although most of the functions of replication have been provided by the host cell. They did not bring one set of nuclear genes that required by the cell for basic growth and division, but they carrying the genes that may be useful on a regular basis which allows cells to exploit the situation of certain environments, for example in the current context, to survive and thrive against antibiotics that potentially lethal to bacteria. Plasmid resistant is any plasmid carrying one or more antibiotic resistance genes (Bennet, 2008). Agarose gel electrophoresis is a technique that works well and is used in clinical laboratories for the analysis of proteins and DNA. This technique uses the principle of zone electrophoresis. As is known, the protein molecules migrate in a solid/gel medium which is soaked with a buffer solution under the influence of an electric field (Giot, 2010).

DNA molecules migrate through small pores are formed in the solid agarose gel. In general, the smaller molecules move faster and migrate farther than large molecules because it has a lower friction when moving towards the positive electrode. Low concentrations of an agarose gel provide better resolution for large fragments, by providing a greater separation between the band in which the size were not too much different. Higher gel concentration, can reduce the speed of migration of large fragments, while it can facilitate better separation at small DNA fragments (Lee & Bahaman, 2010) Therefore, it is necessary to analyze the plasmid profile on E. coli bacteria resistant to meronidazole and nalidixic acid.

2. Materials and methods

a) Study area

This research was conducted from July 2014 to January 2015. The samples were taken in seagrass ecosystems around Tukak, coastal village, in the South Bangka Regency, Bangka Belitung Islands Province (020 58 '09,5' 'SL and 106039'12,7' 'LE) (Figure 1). The Samples were some seagrass species and water substrate.

The samples were hand collecting and then stored in storage box. To analyze dog conch stomach, the inner organs must be separated from the shells, then the inner organs was preserved by using 50% alcohol. The samples were also taken to analyze the consumed preferences and its feeding behavior. The research was performed in laboratory.

b) Procedures

This experiment was carried out by using an aquarium with 60x50x40 cm. The experimental aquarium used glassy separators as a bulkhead for each seagrass species (Figure 2). This experiment had two treatment by using substrate of natural habitats and without using substrate, each treatment was done in three repetitions. These experiments using six seagrass species such as Enhalus acoroides, Cymodocea serrulata, Halodule uninervis, Halodule pinifolia, Halophila minor, Halophila ovalis and six individuals of dog conch. To find the food consumed, there were conducted several stages according to Zupo et al (2001) references: 1) starvation of the dog conch for 24 hours, 2) weighing each seagrass species to initial biomass, 3) treatment; put the seagrass grown of dog conch in every barrier aquarium and 4) the final biomass weighing of seagrass was to determine the plant consumed. In additional, seagrass was calculated to quantify and characterize the seagrass leaf morphologically before and after the treatments. The results of these experiments to determine the preferences of dog conch on seagrass species consumed.

Cultivation of E.coli esistant to metronidazole and nalidixic acid were using biphasic media Brain Heart Infusion (BHI) that BHI plus liquid). One colony of bacteria from media Mac Conkey was inoculated into 50 ml BHI medium, then incubated at 370 C for 24 hours with agitation, after which the culture was used as a starter put in 300 ml BHI medium liquid and then incubated at 370 C for 3 hour with agitation, subsequently incorporated into the culture medium BHI agar slant (in bottles flat), each bottle of 15-20 ml bacterial culture, incubated 48 hours at a temperature of 370 C without agitation, the culture is ready for harvest.

c) Plasmid isolation

Cells were harvested using 1.5 ml eppendorf tubes, centrifuged at 1200 rpm at 5 minutes, repeated twice and subsequently isolated plasmids. Pellets result centrifugation resuspended with 200 µl of lysis solution 1. Over a period of 30 minutes, into the suspension is added a solution of 2 500 µL lysis and left for 5 minutes. III lysis solution was added to the suspension and after settling for 30 minutes, the suspension is centrifuged at 12,000 rpm for 5 minutes. The obtained supernatant was transferred into a new eppendorf tube, plus phenol chloroform isoamyl alcohol (CCIA) (1:1) by volume supernatant, as well as in the vortex for 1 minute and then centrifuged again 12000 rpm for 5 minutes. Plasmids in the supernatant were transferred to a new eppendorf tube.

Before precipitated at -80 ^{o}C temperature for 30-60 minutes, a solution of plasmid M Na. acetate (1/10 volumes of supernatant) and absolute ethanol (1:1). Plasmid solution was washed with how centrifuged 12000 rpm for 5 minutes, and drained. Plasmid (sediment) plus Tris-EDTA (TE) buffer. Plasmid purification is done by adding 10 mL of RNase (10 mg / lt) 37^{o} C incubated for 1 hour, added phenol, divortex for 1 min and centrifuged at 12,000 rpm rotational speed for 5 minutes. Plasmid solution are taken and put a new eppendorf tube plus 3 M Na acetate (l/10 vol.supernatan) and plus absolute ethanol (l: 1), -70 ^{o}C incubated for 1 hour. Plasmid solution was further centrifuged 12000 rpm for 5 minutes. The supernatant was discarded and the precipitate (plasmid) was washed with 70% ethanol and then centrifuged back. Once it dried the TE buffer were added and stored at 4 degrees before electrophoresis.

d) Separation

The plasmid obtained is observed by agarose gel electrophoresis. Agarose

concentration was 1% and the DNA was taken as much as 5 mL and mixed with 5 µL loading buffer. 8 µL as the DNA molecular weight markers were added. Electrophoresis with Tris-borad EDTA (TBE) buffer pH 8.0 is run with an electric potential of 110 V for 60 minutes until a blue color mark bromfenol reach the end of the gel line mark. The stained plasmid will be seen as fluorescent DNA bands (fluorescence) by ultraviolet light.

e) Data analysis

The obtained data were analyzed using descriptive analysis.

3. Result

The test results in resistance to antibiotics metronidazole and nalidixic acid from the overall sample showed mostly E. coli shows the results resistant to both antibiotics. This resistance indicates that E. coli originating from nature or from humans have been resistant to metronidazole and nalidixic acid.

The DNA plasmid isolated from bacteria resistant to antibiotics metronidazole and nalidixic acid. The plasmid isolation results then separated on agarose gel. The results of the separation on agarose gel is as follows:

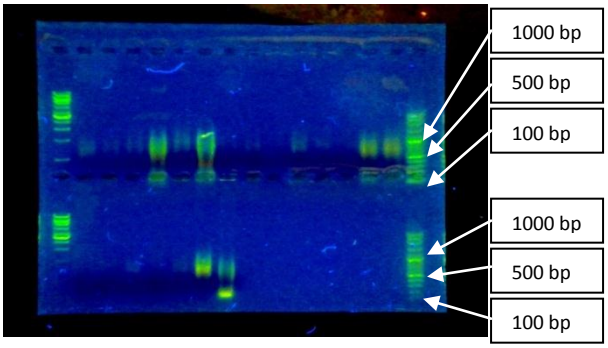

Figure 1. Agarose separation result

DNA ladder marker 1 KB to 100 bp sized was used to expand the size of the coverage of base pairs that can be known from the bacteria. The results of running on agarose shows similarity in the overall size of the base pairs of samples of bacteria which is resistant to metronidazole and nalidixic acid were measured and resulted approximately 500 bp. Different result shown in sample (control sample) E. coli bacteria that sensitive to metronidazole and nalidixic acid. The sample has the smallest base pairs measurd in 100 bp.

4. Discussion

DNA size can be determined by using electrophoresis and DNA molecular weight markers that have been determined. DNA marker serves as a benchmark in order to know the approximate size of the sample DNA. These were negatively charged DNA molecules, so that it will migrate through the agarose gel towards the positive pole. The larger the size of the molecule, the lower the rate of migration. (Magdeldin, 2012)

Bacteria with multiple antibiotic resistant can spread their antibiotic resistant genes to strains of the same species or to another species or genus through a different mechanism. The mechanism is mainly used is the plasmid conjugation-R. Genetic analysis showed that the conjugation of an antibiotic-resistant isolates had a lot of variety R plasmid that can be distinguished based on molecular size and restriction profile (Pignato, 2009).

Quinolone antibiotic resistance due to the increase of mutations caused by recombination of genes that cause bacterial DNA damage repair, as a result of these antibiotics work. DNA damage caused by quinolone class of antibiotics can be repaired by using the bacteria nucleotide excision repair and homologous recombination. The analysis suggests that not all bacterial resistance genes located on a plasmid, but could be due to a mutation in the gene level in the bacterial chromosome (Cirz, 2005).

Plasmids carrying the types of genes that encode highly diverse functions for sensitivity or resistance to antibiotics. Plasmid isolation results conducted by (Warganegara, 2005) showed E. coli clinical isolates either sensitive or resistant to amoxicillin-clavulanate containing plasmids with a size smaller than 6.7 kb. The results of this study support the

notion of Thomson and Amyes (1992), Saves et al. (1995) and Siu et al. (1998) that sensitivity and resistancy to β-lactam antibiotics in E. coli is generally encoded by genes that are mostly located on the plasmid.

Determinant of sensitivity to antibiotics is plasmid Plasmid would show different results on each bacteria (Widodo, 2007) and in accordance with similar research conducted earlier stating that the plasmid DNA profile enterobacter group of bacteria, namely Salmonella which show resistance to several antibiotics. The Salmonella have plasmids with varying sizes, namely 3.1 to32 Kbp. The research data show that Salmonella was showing resistance to certain antibiotics (may be more than one kind of resistance) and plasmids isolated in varying in sizes (Al-Bahry, 2000).

So it can be said that different bacterial isolates with varying sizes allows the bp size are also different. The sensitivity pattern of bacteria to antibiotics is the same in each region can vary because each region has a different sensitivity patterns and vary at different times and places.

5. Conclusion

Based on the data from this study can draw the conclusion, that E. coli shows resistance to metronidazole and nalidixic acid obtained from feces, well water, ice cubes and ketchup show plasmid DNA profile of E. coli observed as a fluorescent DNA band due to ultraviolet rays. In general, isolated plasmids having similar size measured in 500 bp different with bacteria that sensitive to metronidazole and nalidixic acid antibiotics which has a smaller size measured at 100 bp.

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