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A Study for Antimicrobial Susceptibility of Wetlands to Eliminate Toilet Bacteria

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Abstract

Purpose: The purpose of this study is to investigate whether wetland has antimicrobial activity on pathogenic bacteria in the toilet bowl. **Research design, data and methodology:** Air-dried mud obtained from "Jilmoe Bog" wetland was packed and dissolved in the autoclaved saline. Antimicrobial susceptibility was assessed against three Gram-negative bacteria using disk diffusion method and broth dilution method. Identification of specific bacterium presented in wetland supernatant was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. **Results:** Incubation of three Gram-negative bacteria with wetland supernatant inhibited bacterial growth of the bacteria, otherwise increased prevalence of specific bacterium. It was confirmed that Pseudomonas putida was presented in wetland supernatant. **Conclusions:** The results presented in this study might provide the possibility to utilize wetland supernatant as a bioremediation of toilet bowl bacteria.

Keywords : Antimicrobial susceptibility, Wetland, Disk diffusion method, Broth dilution method, Toilet hygiene

JEL Classification Codes : I00, I10, I18, I30

1. Introduction

Public toilet is the place that a variety of microorganisms inhabit, derived from saliva, skin, urine and feces from human. In particular, a large number of bacteria derived from the toilet bowl still remained despite repeated flushing and detected in aerosol produced by flush toilets for a long time (Johnson et al., 2017). These microorganisms form biofilms called "yellow stain", which make toilet seem to be messy and cause opportunistic infection to human. For example, Barker and Jones demonstrated about the possibility of some pathogenic Escherichia coli infection, which has been induced by aerosol contamination after flushing a domestic toilet (Barker & Jones, 2005). Therefore, preventing the accumulation of bacteria in the toilet bowl by inhibiting growth of harmful bacteria or biofilm formation is important in toilet hygiene.

Many studies have been tried to prevent the accumulation of bacteria in the toilet bowl by adding chemical agents in flushing water. Despite of their excellent performance in antimicrobial and antibiofilm activity, these surfactants are not environmentally friendly and sometimes trigger respiratory/dermal irritants to human (Ivankovic & Hrenovic, 2010, Bello et al., 2009). Therefore, investigation

of alternative methods to prevent prevalence of pathogenic bacteria in public toilet is still required.

Wetlands play an important role in ecosystem because of their high productivity, nutrient recycling and act as carbon sink to regulate greenhouse gas (Bodelier & Dedysh, 2013). Particularly, mud in the wetlands showed antimicrobial activity against several microorganisms, which contributes to the self-purification ability to purify pollutants introduced from harmful bacteria (Pipite et al., 2022). For this reason, some trials have been made to apply the self-purification of mud in wetlands to the sanitation field, however, investigation of removing bacteria living in the toilet using self-purification of wetland ability has not been studied yet.

This study is to investigate whether the supernatant from the wetland in Korea could exert antimicrobial activity on several bacteria which are known to inhabit in the public toilet, which might be considered as an alternative supplement against chemical surfactants.

2. Literature Review

2.1. Wetland Ecosystem and Benefits

Biogeomorphic wetlands cover approximately 1% of the Earth and have played an important role for mankind for a long time as a source, repository, and incubator of change for many of the Earth's chemical, physical factors (Temmink et al., 2022). Because of its contribution to ecosystems by self-purification which degrades organic and inorganic compounds generated by human behavior, wetlands sometimes described by the term "nature's kidney", and its application to public hygiene is environmentally friendly and low-cost approach to prevent the accumulation of harmful organic compounds (Xiang et al., 2018).

2.2. Bacteria Living in the Toilet Bowl

Toilet bowl contains variety of bacteria mainly derived from human materials (body fluids, skin and feces) or contaminated sink water. Previously described in literature demonstrates about some pathogenic bacteria living in the toilet bowl. Escherichia coli (feces), Shigella spp. (feces), Enterococcus spp. and Pseudomonas spp. (contaminated sink water flush), and Staphylococcus spp. (skin) (Abney et al., 2021). These bacteria induce opportunistic infections in toilet that trigger multiple diseases (Gerba et al., 1975).

3. Research Methods and Materials

3.1. Bacterial Culture and Identification of Bacteria Cultured from the Wetland

By literature review, three represent types of bacteria commonly observed in the toilet bowl were selected to investigate antimicrobial activity of supernatants from the wetland. Escherichia coli (E. coli), Enterococcus. cloacae (E. cloacae) and Pseudomonas aerusinosa (P. aerusinosa) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). E. coli, E. cloacae, and P. aerusinosa were cultured in MacConkey agar (Becton-Dickinson biosciences, Braintree, MA, USA) under the humidified atmosphere at 37°C. The mud used in this study was obtained from "Jilmoe Bog" located in Odea Mountain National Park (Pyeongchang, Korea). To make the mud supernatant, mud was air-dried for 48 hours and packed into water-permeable paper. The mud package was then incubated with autoclaved saline for 24 hours to make sure that the ingredients in the mud dissolved well in the saline, followed by filtering with 3M paper for another 24 hours. Identification of bacteria from the mud supernatants was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker, Billerica, MA, USA).

3.2. Examination of Antimicrobial Susceptibility of Supernatants in Wetland

To examine antimicrobial activity, Kirby-Bauer disk diffusion method was performed. Bacterial supernatants were diluted in autoclaved saline by Mcfarland turbidity standard 0.5 and inoculated in Muller-Hinton agar (Becton-Dickinson biosciences, Braintree, MA, USA). Punched paper disks impregnated with different amounts of supernatants in wetland (1 and 10 μL) were placed in appropriate distance. The bacterial plates were incubated in 37°C CO2 incubator for 24 hours and zone of inhibition was examined. For broth dilution method, bacterial suspensions diluted in autoclaved saline by Mcfarland turbidity standard 0.5 were mixed with supernatants from wetland (diluted in 1:10) and incubated in room temperature for 24 hours. Then 1:10 serial dilution was performed to distinguish viable bacterial colonies.

4. Research Results

To investigate whether the supernatant from wetland has antimicrobial capacity on toilet bacteria, three representative types of microorganisms observed in toilet bowl such as Escherichia coli, Enterococcus cloacae and Pseudomonas aerusinosa were examined by disk diffusion method. It was confirmed that zone of inhibition was not found, which

indicates that small amount of supernatant from wetland did not show any antimicrobial activity.

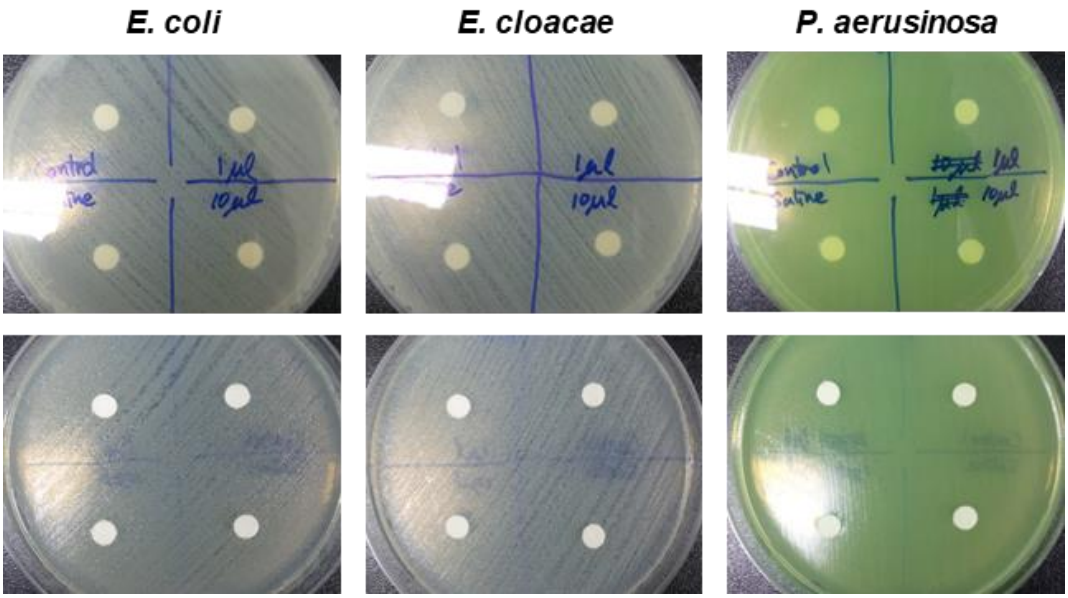

Figure 1: Effects of supernatants from wetland against three bacteria by disk diffusion methods

Next, broth dilution method was performed to examine whether the large amounts of supernatant from wetland showed antimicrobial activity. Unexpectedly, bacteria incubated with supernatant from wetland increased the number of bacterial colonies. Especially, apart from the three Gram-negative pink colonies which was known to lactose fermenters, yellowish colonies were observed in the MacConkey agar which indicates that specific bacterium was presented in the supernatant from wetland.

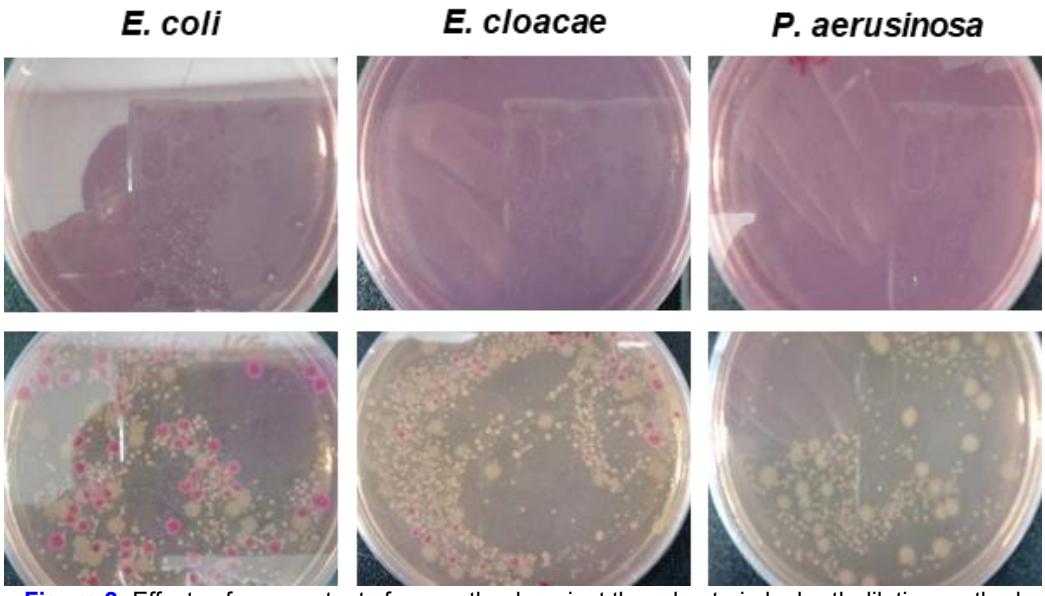

Figure 2: Effects of supernatants from wetland against three bacteria by broth dilution method

Therefore, investigation of which microorganisms are present in the supernatant from wetland was processed. Result of figure 3 showed that some specific yellowish colonies were observed in MacConkey agar inoculated with

the supernatant, and it was confirmed by MALDI-TOF MS analysis that Pseudomonas putida was presented in the wetland supernatant. Considering that this bacterium was cultured more than directly inoculated bacteria, P. putida decreased the prevalence of other bacteria, and which might indicate that P. putida inhibited growth of three Gram-negative bacteria.

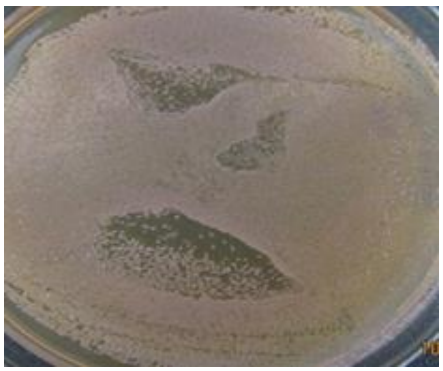

Figure 3: Bacterial culture of wetland supernatant in MacConkey agar

5. Discussion

In recent years, bioremediation using environmental bacteria has been widely applied as an alternative method to prevent abuse of chemical agents because of its environmentally friendly and safety for mankind. Bacteria possess metabolic processes that could degrade pollutants/organic wastes derived from human behaviors which are mainly composed of carbon, nitrogen and sulfur (Azubuike et al., 2016). In addition, some beneficial bacteria can inhibit other bacteria which have been known to induce pathogenic infection in human, by the secretion of metabolites (Mendez Garcia & Garcia de Llasera, 2021). These metabolites are not only to prevent the pollution of the environment, but also purify the polluted environment.

Microbiome in wetlands contributes to biological processes of wetlands including carbon cycle (methanotrophs) and ammonium oxidation (Graef et al., 2011, Ferousi et al., 2017). Therefore, many studies have been demonstrated to utilize microbial activity in wetland ecosystem. Constructed wetland-microbial fuel cells combine microbiota are used to transform chemical energy into electricity by oxidating organic matter (Wang et al., 2020). Microbiota in constructed wetlands have been investigated as an alternative to treat in wastewater (Pacheco Aguilar et al., 2008). Moreover, Bacillus sp. isolated from wetland could inhibit in human pathogens, which might be applied in hygiene area (Cavalini et al., 2021). These applications using microbiota in wetlands have been introduced due to its advantages in that of efficacy, low-cost, environmentally friendly.

Pseudomonas putida is a Gram-negative bacterium prevalent in soil and water, particularly in the rhizosphere at a relatively high population density (Peter et al., 2017). This microorganism has been reported that it has capability of bioremediation of aromatic compounds such as naphthalene and styrene, which is predominant in the polluted area (Pozdnyakova-Filatova et al., 2020). Especially, a study about the antimicrobial potential of P. putida revealed that several strains of P. putida inhibited biofilm formation and growth of pathogenic bacteria (Babich et al., 2021, Marinho et al., 2009). This results partially consistent with our results that prevalence of P. putida from wetlands was dominant compared with other bacterial growth, which indicates that P. putida might inhibit growth of some pathogenic bacteria derived from human behaviors. In order to apply it to real life, it is thought that further experiment should be conducted to investigate whether the growth of toilet bacteria is inhibited when the supernatant isolated from wetland is applied to toilet flush water.

This study has some issues of limitation that investigation of supernatants from wetland was performed in one area. The wetland bacteria vary with depend on the environment, particularly on the types of wetland sediment and the climates (Bodelier & Dedysh, 2013). In addition, despite the wetlands contain verity of bacterial communities, but this study identified a single bacterium in the wetland. Although there are complications to identify a large number of bacteria at once with the existing classical bacterial culture method, recent methods such as whole genome 16S ribosomal RNA-sequencing analysis based on next-generation sequencing might provide a large number of bacteria at a time from small amount of samples (Church et al., 2020). Therefore, further studies to identify diverse of microorganisms inhabiting wetlands in other regions and additional research on their antimicrobial activity against the toilet bacteria are required.

6. Conclusions

Results of this study demonstrated that specific bacterium from the wetlands in Korea, P. putida inhibited growth of other pathogenic bacteria. It was confirmed that incubation of three Gram-negative bacteria with wetland supernatant inhibited bacterial growth of the bacteria,

otherwise increased prevalence of specific bacterium presented in wetland supernatant. These results might provide the possibility of utilizing wetland supernatant as a bioremediation of toilet bowl bacteria.

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