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Human-associated *Escherichia coli* Marker: Important Indicator to Evaluate River Water Quality and Treatment Ability of Surrounding Wastewater Treatment Plants

Pimchanok Nopprapun*, Suwanna Kitpati Boontanon*', Shigeo Fujii** and Hidenori Harada**'***[*]**
*Civil and Environmental Engineering Department, Mahidol University, Nakhon Pathom, 73170, Thailand
**Graduate School of Global Environmental Studies, Kyoto University, Kyoto, 606-8501, Japan
***Graduate School of Asian and African Area Studies, Kyoto University, Kyoto, 606-8501, Japan
[*]E-mail : harada.hidenori.8v@kyoto-u.ac.jp

Abstract

River water contamination can threaten human health because waterborne pathogens in environmental water can expose to humans via recreational activities. In addition, river water is an important source for water consumption in Thailand. Therefore, microbial source tracking has become a crucial method to evaluate the source of fecal contamination, water quality, and to understand the cause of fecal contamination by investigating the treatment ability of surrounding wastewater treatment plants (WWTPs) affecting the river water quality. In this study, 200 *Escherichia coli* isolates collected at four sampling sites around WWTPs of the Mae Klong River were tested with a human-associated *E. coli* genetic marker (H8) for evaluation the human fecal pollution in the river. Moreover, water quality parameters were measured at the same sites. The levels of H8 marker detection were also investigated with the ability of WWTPs among four sites along the river. Real-Time PCR was performed on the isolated samples with the H8 marker and the results showed that proportions of positive isolates increased at Mueang Kanchanaburi district (KP1: 46%) and Mueang Ratchaburi district (RP1: 38%). The positive proportions from all sites were significantly different between the locations ($p < 0.001$). Furthermore, the number of population and wastewater treatment plants capacity were relatively high in those areas (KP1 and RP1). This study showed that the H8 marker can be used as a crucial genetic marker for identifying the human-source contamination in water environment. Furthermore, it can be suggested that management of wastewater treatment system is a key to reduce human fecal pollution for better environmental water quality.

Keywords : Microbial source tracking; Human-associated genetic marker; *E. coli*;
Wastewater treatment plants; Thailand

Introduction

Pathogenic bacteria can expose to environmental water via human and animal feces from wastewater overflow of wastewater treatment plants, septic tanks, and urban runoff [1]. Contact with waterborne pathogens from environmental water can occur accidentally from swallowing of contaminated feces during recreational activities such as boating, swimming, or through consumption of undercooked aquatic animals [2]. Many different types of pathogens may exist in aquatic environment and it is time-consuming, cost prohibitive, and impossible to screen all of pathogenic bacteria from water samples [3]. Therefore, worldwide regulators normally measure fecal indicator bacteria (FIB) such as *E. coli* and enterococci in surface water as a representative of pathogens for many years [4]. In addition, FIB is prevalent in animal feces so it does not provide information about specifically contaminated sources [5].

River in Thailand receives wastewater from point and nonpoint sources such as agricultural runoff, industries, urban runoff, and domestic wastewater. Downstream area of the river is massive in terms of water usage [6]. Recently, a human-associated genetic marker for *Escherichia coli* (H8) has been developed and successfully used to evaluate the human source from human-associated *E. coli* in drinking water and water environment [7-9]. *Escherichia coli* are a gram-negative bacteria (GNB) which can be found in intestinal of a variety of animals and humans. Not all of *E. coli* strains are harmless but some of them can cause fatal diseases in humans [10]. Thus, microbial source tracking has become an important method to understand the source of fecal contamination and to decrease the hazard of waterborne diseases that occur in environmental water. In this study, four sampling sites of the Mae Klong River were investigated the river water quality which were surrounded by wastewater treatment plants in Thailand. In addition, the H8 marker was applied as a suitable method to evaluate the human source from fecal contamination at the same sites. Moreover, the treatment ability of surrounding wastewater treatment plants was investigated with the H8 marker to understand the cause of fecal contamination in the river and be able to suggest proper water quality management.

Methodology

Collecting river water samples

River water samples were collected from ten sampling sites along the river but four sampling points were focused for this study to evaluate the treatment ability at WWTPs of each city affecting the river water quality at KP1 (Mueang Kanchanaburi), RP3 (Ban Pong), RP2 (Photharam), and RP1 (Mueang Ratchaburi) districts [11]. 1,000 mL of river water samples at each of sampling site were collected in sterilized bottles at the center of water flow. Furthermore, dissolved oxygen (DO), potential of hydrogen ion (pH), electric conductivity (EC), temperature, turbidity, and total organic carbon (TOC) were measured for water quality measurement at the same sites. All samples were transported in a cooling box, kept it in the dark at 4 °C to the laboratory, and processed within 12 hours.

Culturing and collecting *E. coli* isolates

River water samples were filtrated by using 100 mL sterilized funnel and disposable filtration devices (0.45 µm, white MCE membrane, Merck, Germany). Then, the filter was placed on HiCrome Chromogenic Coliform Agar (recommended for *E. coli* detection, HIMEDIA, India) and the petri dishes were incubated at 37 °C for 22 hours. For *E. coli* analysis, blue colonies of *E. coli* were picked up by using sterilized toothpicks and transferred it into each well of a 96-well plate filled with 50 µL MilliQ water. These 96-well plates were stored and kept at -20 °C for a maximum of 24 hours to PCR analysis.

Conducting Real-time PCR

For PCR analysis, *E. coli* isolates were performed by using SYBR Green based Real-time PCR assays with the H8 marker for detecting human fecal pollution. In the total of 15 µL of PCR mixture, it was composed of 7.5 µL of QuantiFast SYBR green PCR (QIAGEN, Germany), 4.9 µL of MilliQ water, 2 µL of *E. coli* samples, and 0.3 µL in each of forward and reverse primers. All of PCR reactions were performed on a 96-well plate for Real Time with Thermal Cycler Dice Real Time System (BIO RAD, Singapore). Positive (DNA from control strains) and negative (MilliQ water) controls were included for each PCR assay. The Real-time PCR conditions were set at 95 °C × 5 min + (95 °C × 10 sec + 60 °C × 30 sec) × 40 cycles + melting curve analysis. The H8 genetic primer sets were used for SYBR Green based PCR assays are shown the primer and target DNA sequence in Table 1.

In addition, wastewater treatment plant locations in the research areas were obtained from Pollution Control Department [12].

Results and Discussions

Transition of water quality along the river

Figure 1 shows the results of river water quality from upstream to downstream along the Mae Klong River which were surrounded by wastewater treatment plants. The transition of pH along the Mae Klong River was quite stable values. It was neutral around 7. Temperature from all sampling sites was around 28.3 to 29.6 °C. For conductivity, turbidity, and TOC, it increased gradually along the river flow and higher than other sampling sites, especially at RP1 (conductivity: 247 µS/cm, turbidity: 12 NTU, and TOC: 2.651 mg/L). This sampling site showed that river water quality was affected by the accumulation of organic substances from the river flow. However, DO gradually decreased along river flow which was consumed by photosynthetic organisms by high levels of organic pollutants. The lowest value of DO was in RP1 (DO: 2.4 mg/L). According to water quality standards of the surface water in Thailand, the Mae Klong River was in Class 3 which was mainly used for consumption and agriculture but it has to be passed through an ordinary treatment process (Class 3 standard: DO > 4 mg/L, BOD < 2 mg/L, Fecal Coliform Bacteria < 4000 MPN/100 mL). DO along the Mae Klong River in this study do not pass and meet the surface water quality standards in Thailand [13]. Furthermore, National Institute of Information Technology Water and Agriculture reported that river water quality in some stations of the Mae Klong River was likely deteriorated. The contamination of total coliform bacteria and fecal coliform bacteria was quite high because wastewater was largely discharged from the excretion of households and communities into the river [14].

Table 1 Primer sets for SYBR Green based PCR assays

Gene	Name	Primer sequence	Target DNA sequence	Product size	Source
H8	H8-F	ACAGTCAGCGAGATTCTTC	ACAGTCAGCGAGATTCTTC	177 bp	[7]
	H8-R	GAACGTCAGCACCACCAA	TTGGTGGTGCTGACGTTC		

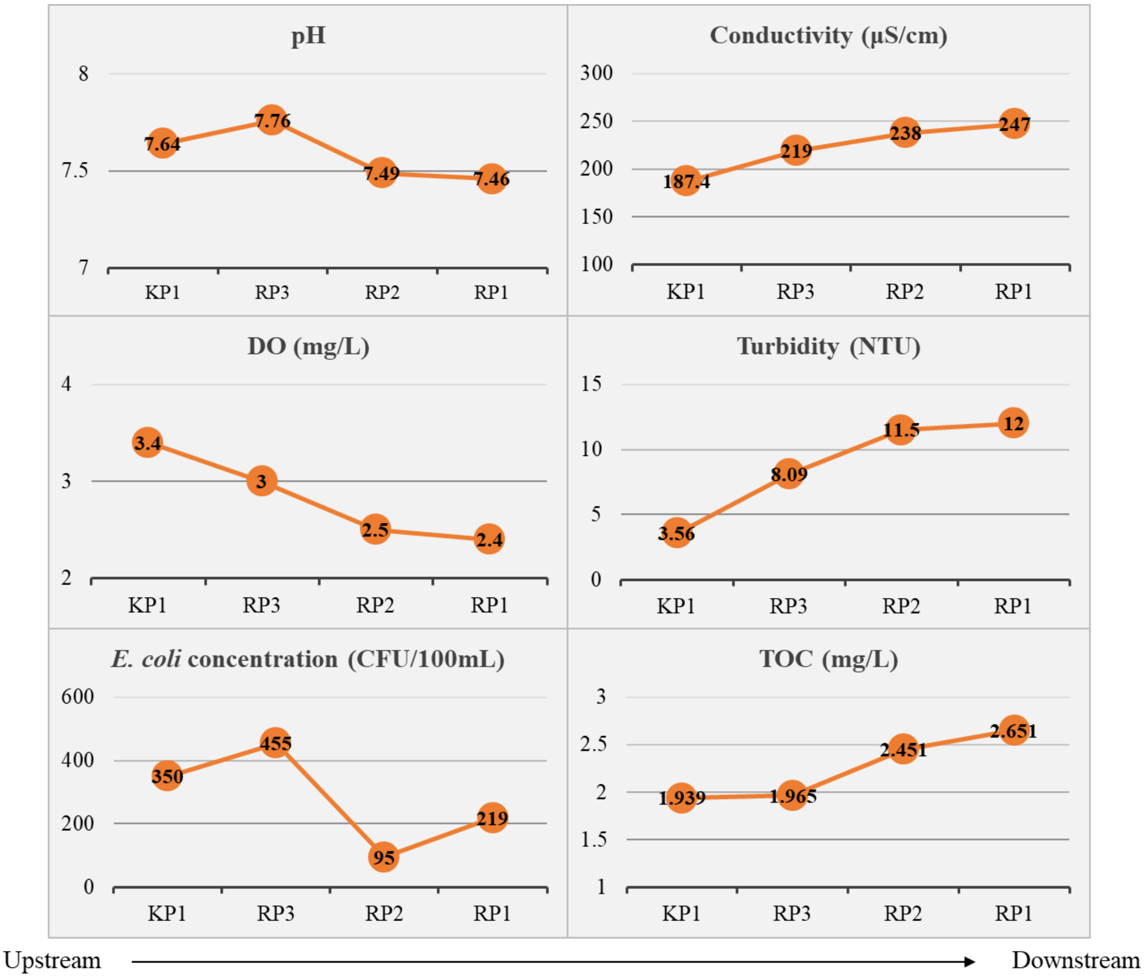

Figure 1 The transition of river water quality from upstream to downstream along the Mae Klong River

The results of conductivity, turbidity, and TOC concentration showed that the pollutants concentration increased, especially in downstream areas of the Mae Klong River. In addition, *E. coli* concentration indicated that microorganisms were high levels of fecal contamination, especially at KP1 and RP3. However, the contamination results of other water quality parameters were not similar trends with *E. coli* concentration. Those water quality parameters were high values and increased continuously along the river flow but *E. coli* represented the maximum values at some sampling sites. Therefore, the water quality parameters showed that it was polluted along the river flow which were highly discharged from point and nonpoint sources and accumulated at the downstream areas but the sources of pollutants from human or animals could not be properly identified. Nevertheless, *E. coli* concentration might be largely affected by domestic or animal wastewater contamination at the areas of KP1 and RP3 in Kanchanaburi and Ratchaburi provinces, respectively. According to the results of *E. coli*, it could be seen that there were many interesting sampling points along the river which were found fecal contamination. However, only *E. coli* parameter was not enough to identify the source of human fecal contamination and further study should apply other animal genetic markers to determine other sources of fecal contamination [15-16]. Therefore, this study applied microbial source tracking method which was necessary to find the human source of fecal pollution in environmental water that can affect to human health with the H8 marker.

Human fecal contamination in environmental water

Figure 2 shows *E. coli* and H8 positive percentages from four sampling sites of the Mae Klong River. The results of *E. coli* concentration were 350 CFU/100 mL (KP1), 455 CFU/100 mL (RP3), 95 CFU/100 mL (RP2), and 219 CFU/100 mL (RP1), respectively. Real-Time PCR was performed with the H8 marker for 200 *E. coli* isolates collected at four sampling sites. The results of H8 positive percentages in each sampling site showed that proportions of positive isolates were 46%, 18%, 14%, and 38% at KP1, RP3, RP2, and RP1, respectively. The H8 positive proportions from all sampling sites were significantly different between the locations ($p < 0.001$). Comparison of *E. coli* concentration and H8 positive percentages illustrated that the higher *E. coli*, the higher H8 positive of the Mae Klong River. However, RP3 was found different correlation with positive percentage of the H8 marker. According to the number of livestock in Kanchanaburi and Ratchaburi provinces, it was found that duck, dairy cattle, and buffalo were the most animals number at Ban Pong district (RP3) in Ratchaburi province [17-18]. Therefore, the higher number of animals might affect *E. coli* highly contributed to RP3 from animal feces and high activity of animal farms. From these results, the H8 marker showed that the areas of KP1 and RP1 were more polluted by domestic wastewater discharged to the river. Therefore, the information of the number of population and wastewater treatment plants ability in those areas were needed to describe this situation.

Relation of human fecal pollution and wastewater treatment plants ability for water quality management

Construction of wastewater management projects completed in the Mae Klong River Basin has been operated by Department of Public Works and Town and Country Planning. Table 2 shows details of wastewater management projects completed in the Mae Klong River Basin.

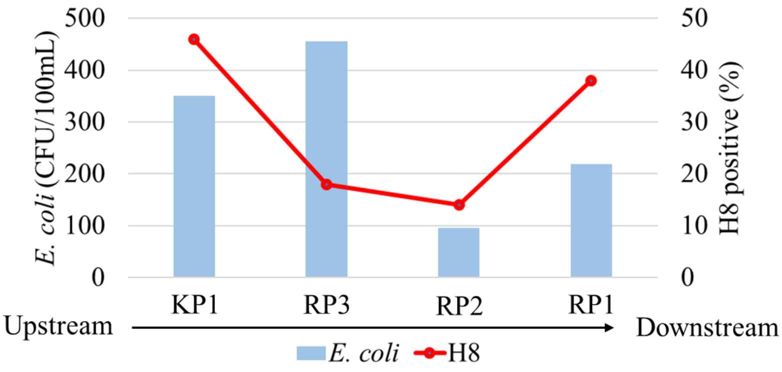

Figure 2 *E. coli* and H8 positive percentages

Table 2 Wastewater management projects completed in the Mae Klong River Basin

River water sampling sites	Provinces	Districts	System	Capacity (m^3/day)
KP1	Kanchanaburi	Mueang Kanchanaburi	Oxidation Ditch	24,000
RP3	Ratchaburi	Ban Pong	Stabilization Pond	5,000
RP2	Ratchaburi	Photharam	Oxidation Ditch	5,000
RP1	Ratchaburi	Mueang Ratchaburi	Stabilization Pond	20,000

These wastewater treatment plants (WWTPs) consist of four locations along the Mae Klong River and covered only 48% of the people in those areas. In addition, all of the capacity can treat domestic wastewater around 54,000 m^3/day [12].

In this study, it was found that human fecal pollution was more problem than other areas, especially at KP1 and RP1. Moreover, it was found that the number of population in those areas were also high at KP1 and RP1 where both are Mueang districts in Kanchanaburi and Ratchaburi provinces, respectively [19]. Figure 3 shows comparison of wastewater treatment plants capacity and H8 positive percentages. According to Figure 3, wastewater treatment plants capacity was projecting the similar trend with the H8 positive percentages which can be implied the number of population in those areas as well. Although, KP1 and RP1 have had high capacity of domestic wastewater treatment plants in the areas, the percentages of human-associated *E. coli* fecal pollution were still high levels. Therefore, the treatment ability of these WWTPs were needed to closely investigate. According to Kanchanaburi Municipality Office, WWTP at KP1 has been constructed since 1995 and operated until now. There is disinfection system (chlorination) in this plant but it has been inefficiently operated. Furthermore, population and urban areas have continuously expanded. Therefore, collection of wastewater from sewerage system to the WWTP has not covered all of the areas and as a result, people have directly discharged domestic wastewater into the river [20]. Furthermore, there is no disinfection process in the WWTP of Mueang Ratchaburi district (RP1) so high volume of wastewater has been discharged with high volume of human fecal pollution to the river [21]. However, the H8 positive percentages and wastewater treatment plants capacity at RP3 and RP2 decreased because lower number of population and volumes of domestic wastewater in that areas where lower discharged to the river than the areas of KP1 and RP1. [22] recently reported that the river water sampling stations which are surrounded by urban areas and high effluent from WWTPs have discharged and increased human fecal pollution levels in the river. Furthermore, a significant increasing of fecal contamination from human mainly due to WWTPs capacity cannot receive an exceedance of the wastewater or individual septic systems continuously discharged [23].

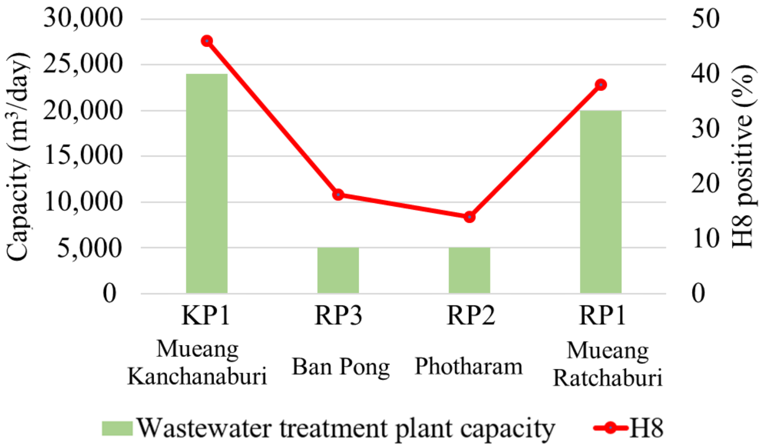

Figure 3 Wastewater treatment plants capacity and H8 positive percentages

These results showed that KP1 and RP1 which are surrounded by urban areas and affected with high levels of fecal pollution. Therefore, suitable capacity of wastewater treatment system with the number of population and appropriate management of septic tank systems are required for better water quality management at Mueang Kanchanaburi and Mueang Ratchaburi districts in Kanchanaburi and Ratchaburi provinces. In this study, the utility of the H8 marker can help to understand the human source of fecal contamination in environmental water. Furthermore, it will be useful for decision maker to implement a robust water quality management plan.

Conclusion

The H8 marker was used to investigate the source from human contamination at the sampling locations which wastewater treatment plants have discharged wastewater to the Mae Klong River, Thailand. The river water quality showed that the pollutants concentration increased, especially in the downstream areas (Mueang Ratchaburi) of the river but *E. coli* concentration were high levels of fecal contamination at Ban Pong district. In addition, the results of H8 positive percentages illustrated that human-associated *E. coli* were largely contributed to the areas of Mueang Kanchanaburi and Mueang Ratchaburi districts where the number of population were high. These results were projecting the same trend with the treatment capacity of wastewater treatment plants implied to the population density in those areas. The additional information of their treatment abilities indicated that wastewater treatment plants in those areas could not decrease human fecal pollution before discharged to the river. Therefore, this study suggested that a human-associated *E. coli* marker could be used as an important indicator to evaluate river water quality and treatment ability of surrounding wastewater treatment plants. Furthermore, management of municipal wastewater treatment plants are significant to limit human fecal pollution in the water environment.

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