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Comprehensive analysis of pathogenic bacteria in environmental samples using metagenomic approaches
（メタゲノム解析による環境中の病原性バクテリアの総合解析）

Waterborne diseases caused by pathogenic bacteria are a concern for public health worldwide. The lack of information of overall pathogens in aquatic environments makes it difficult to establish a strategy for the prevention of pathogen infections from water. Recently, metagenomic analysis combined with the ability of next generation sequencing technology can be used for the direct detection of pathogenic bacteria in water samples. One important process in metagenomic studies is the taxonomy classification of bacteria. Several database search methods have been used for taxonomy classification of bacteria using bioinformatics algorithms, such as blastn searches. In this thesis, the different database search methods for taxonomy classification of bacteria were compared for their diagnostic ability in detecting pathogens. By focusing on a specific bacterium species, *L. pneumophila*, the detection results of each search method were compared with the results from *L. pneumophila*-specific nested PCR. The results demonstrate that database search methods using complete genomes as reference sequences showed numerous false positives and had a potential problem with specificity. On the other hand, database search methods using long specific marker genes as reference sequences provided the best agreement of detection with *L. pneumophila*-specific PCR. Subsequently, database search methods using specific marker genes were selected to analyze pathogenic bacteria in environmental samples collected from urban and rural areas in Thailand. The results indicated that pathogenic species from the genera *Aeromonas*, *Bordetella*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, and *Ralstonia* were common in these samples; they were detected in all samples with high abundance. In addition, the profiles of antibiotic resistance genes (ARGs) in each sample group were analyzed using a blastn search against the ARG database which contains reference sequences of ARGs. The results showed that the abundance of reads identified as ARGs in samples collected from urban areas were higher than those in samples collected from rural areas; indicating that urban environments may serve as reservoirs for the circulation of antibiotic resistant bacteria in the environment. In conclusion,

metagenomic analysis could be a useful tool to establish a strategy for the prevention of waterborne diseases.