Efficient DNA extraction method and estimation of bacterial number by real-time PCR from environmental water samples

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Recently, the research in microbiology has accomplished rapid progress along with the development of molecule biological techniques. Especially, the quantification and community analysis of microbes by using molecule methods have been applied, because they are simpler and quicker techniques in comparison with the analysis by conventional cultivation methods. Microbes are present in any environment including groundwater, river, sediment and soil. They play a role in improving and changing the environment through material cycle. On deep geological environment, microbes affect the changes in the redox of groundwater which is one of the most important properties to estimate the condition of a geological disposal system. It is necessary to quantify microbial biomass on the geological environment to evaluate the redox due to microbial activities. We compared to DNA extraction methods from environmental water samples. Then, we establish a quantitative analysis by real-time PCR with SYBR Green for domain bacteria.

The water samples obtained from Horonobe borehole HDB-10(depth ca.-500m), river A and river B. The liquid cultures of *Escherichia coli* also used as a control experiment. We compared three DNA extraction methods, two beads beating methods (method A, method B), and one enzymatic lysis method (method C). The DNA extraction methods were evaluated by the measurement of quality and yield of DNA and by a convenience in handling. To evaluate the quality of the extracted DNA, absorbance ratios at 260 nm/230 nm (DNA/humic acids) and 260 nm/280 nm (DNA/protein) were determined using spectrophotometer. The DNA yield was evaluated by Ct value in real-time PCR. High yield of the DNA by the method A was obtained within a relatively short time. The reproducibility of method A was high. This method was able to prevent the DNA loss on handling due to having a low number of steps of DNA extraction. Therefore, the method A was suitable for extract DNA from environmental water samples.

The measurement of domain bacteria in water samples consisted of quantification of DNA concentration using real-time PCR and conversion from DNA concentration to viable cell number of *E. coli* . A SYBR Green real-time PCR assay was developed making use of serial dilution of *E. coli* genomic DNA. The domain bacteria specific primer pair com1/com2 was used in this real-time PCR assay. (Schwieger and Tebbe, 1998, Zhou et.al., 2007). The results of real-time PCR at the DNA samples from Horonobe groundwater, river A and river B using the extraction method A was 483-700, 13696-20000 and 51824-290800 ng /L water, respectively. The converted value from DNA concentration to viable cell count number of *E. coli* at Horonobe groundwater, river A and river B was 5.12-7.42 x 10^3, 1.45-2.12 x 10^5 and 0.55-3.08 x 10^6 cfu/ml, respectively. The conversion was conducted based on the correlation (y=0.0106x, R2=0.9908) of viable cell number of *E. coli* on plate medium and DNA concentration extracted from this *E. coli* . In this way, the converted value at *E. coli* liquid cultures was 2.00-4.13 x 10^8 cfu/ml. This converted value was almost the same number of *E. coli* liquid cultures (1.29 x 10^8 cfu/ml). This result suggests that this real-time PCR assay will be useful for the quantification of domain bacterial DNA concentration and the estimation of converted viable cell number of *E. coli* from environmental water samples.

This research was performed by