

Change of microbial community in groundwater after the 2016 Kumamoto earthquake

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Introduction: Kumamoto City is the largest groundwater utilization region in Japan. About 0.75 million people in the city depend entirely on groundwater as the source of drinking water. The Kumamoto groundwater studying area was defined mainly two lines. One is the flow line from Mount Aso to the Ariake Sea through Lake Ezu (A line), and the other is the line from Ueki highlands to the the Ariake Sea through Shira River (B line). There exit hotspots of nitrification in the middle area of B line, where concentration of nitrate decreased drastically. After the occurrence of continuous Kumamoto earthquake on April 2016, not only the physicochemical parameters but microbial community would be changed. In this study, microbial communities of total bacteria and denitrifying bacteria in Kumamoto groundwater after earthquake were analyzed and compared with those before earthquake.

Materials and methods: Four samples (B-S, B-IS, B-ID, A-W) were collected from 2 sites (B-I, B-S) in B line and 1 site (A-W) in A line. B-I and B-S are located inside of denitrification hotspot, while A-W as a control is outside of the hotspot. The extraction of total DNA from 4 filtered samples was performed with the PowerWater Sterivex DNA Isolation Kit (MO BIO). Partial bacterial 16S rRNA and *nirS* genes were amplified with 27F/518R and *nirS*-1F/6F primer sets, respectively, during PCR amplification. After PCR products were ligated with T- vector and transformed into *Escherichia coli* DH5 α , plasmid extraction was subsequently performed. Restriction enzyme set *EcoRI*/*PstI* were used to verify the positive clones for sequencing. These obtained clones and their nearest strains by BLAST were used to perform phylogenetic analysis.

Results and discussion: It was clear that β -Proteobacteria decreased while Actinobacteria increased after earthquake. Specifically, α -Proteobacteria and β -Proteobacteria account for 23% and 32% of total sequences, respectively, before earthquake. Their ratios reduced to 6% and 17% after earthquake. On the contrary, the ratio of Firmicutes and Actinobacteria rose from 15% and 1% to 29% and 13%, respectively. In the genus level, the microbial variety performed large distinction before/after earthquake. For example, before earthquake, methylophilic bacteria such as *Methylophilus*, *Methylobacter*, *Methylothermus* and *Methylobacterium* were widely distributed in groundwater samples. However, the salt-tolerant bacteria and general bacteria in human life were found in the period of post-seismicity. The *nirS* gene of denitrifying bacteria could not be amplified after earthquake, although it was strongly amplified before earthquake. The isolated denitrifying bacteria from sample B-IS/ID and B-S were (per)chlorate-reducing bacteria *Dechlorosoma suillum* PS and autotroph *Sulfuricella denitrificans*-like bacteria. The obtained bacteria such as (per)chlorate - reducing bacteria in the groundwater offered one possibility that the contaminants passed through the aquifers due to breakage of aquiclude caused by earth shock. Despite of low number, the existing data still can provide some clues for insight into the effects of earthquake on the microbial community in subsurface environments.

Keywords: groundwater, microbial community, clone analysis