Monitoring Impact of Oil Biostimulation Treatment on Indigenous Bacterial Communities by Dgge and Clone Library Analyses

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Biostimulation is expected to be an effective alternative tool for residual oil cleanup after physical and chemical removal of oil on rocky shorelines, commonly observed in Japan, where such treatments are not effective. However, there are few studies on the impact of biostimulation on the ecosystems. Furthermore, determination of safety for human health of the bacterial strains that became dominant, and of restoration of the bacterial community structure after biostimulation could provide valuable information on the risk assessment of this method. On the other hand, it is difficult to culture most bacteria in environmental samples, evaluation of the changes in the bacterial community structure only by culturing is inadequate. Hence, changes in the bacterial community structure during field experiments on spilled oil biostimulation were monitored by denaturing gradient gel electrophoresis (DGGE) and clone library methods based on 16S rRNA gene (rDNA) sequences to assess the impact of biostimulation. The field study was carried out on the Sakondani Coast, Hyogo pref. in Japan from 1998 to 1999, which was polluted by spilled crude oil from the tanker "*Nakhodka*" in 1997. The small rocks contaminated with crude oil were piled up, and Inipol EAP22, which is oleophilic liquid fertilizer containing nitrogen and phosphorus, was sprinkled every 11 weeks from Jun. 18 to Sep. 1, 1998. Interstitial water samples were collected just before the time of each application of nutrients, and also after the fertilization period. The samples were filtered, and total DNA was extracted from bacterial cells. V3 region of 16S rDNA was amplified and applied to DGGE. The DGGE banding pattern was analyzed with Shannon index of bacterial diversity and principal component analysis (PCA). Nearly complete 16S rDNA was amplified and clone libraries were constructed. About 50 clones randomly selected in each library were sequenced and phylogenetically analyzed. The results of DGGE, coupled with the use of the Shannon index and PCA, and clone library analyses were consistent, and they revealed that the bacterial community structure in the treated (fertilized) area was markedly different from that in the control (non-fertilized) area during the fertilization period but that in the two areas it became similar at 14 weeks after the end of fertilization. In the treated area, one operational taxonomic unit (OTU) became dominant during the fertilization period, and it was most closely related to *Pseudomonas putida* (97% similarity), based on the nearly complete 16S rDNA sequence. This population accounted for about 60% and about 90% of the total population based on the DGGE and clone library methods, respectively. The results suggested that the bacterial community structure was disrupted by the biostimulation treatment but that it recovered immediately after the end of fertilization.