Introduction of Violacein-Producing Genes into Trichloroethene-Degrading Bacteria to Avoid Protozoan Predation

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Background/Objectives. Bioaugmention is an attractive treatment method to cleanup soil and groundwater contaminated. We have developed various kinds of trichiloloethene (TCE)-degrading bacteria to decompose TCE in the field. However, these bacteria could not work long enough to cleanup in the field. Our results showed that bacteria introduced were rapidly eaten up by indigenous protozoa. In order to overcome this problem, we have focused on violacein that is known to have an antiprotozoal activity. We have examined to confer violacein producing ability to TCE-degrading bacteria.

Approach/Activities. Four types of violacein-producing genes, *vioABCDE*, were cloned from four different bacterial strains. Resultantly the genes from *Chlomobacterium violaceum* JCM1249 were found to produce violacein most efficiently in *Escherichia coli* JM109 used as a host. Then the *vioABCDE* was introduced to two types of *Cupriavidus* sp. bacteria that can express phenol hydroxylase gene constitutively and degrade TCE efficiently at low concentrations. The *vioABCDE* were integrated into the chromosome DNA of the bacteria by using a Tn5 transposon vector. Colonies of transconjugants showed violet color due to violacein production. Transconjugants that showed deeper violet color were selected from among transconjugants. Two selected transconjugants derived from each bacteria strain were used for batch experiments to examine their abilities to avoid protozoan predation and to degrade TCE. The bacterivorous flagellate, *Spumella* sp. TGKK2 (NBRC 111014), that is a kind of protozoan often observed in the natural environment as a predator of bacteria, was used for predation experiments.

Results/Lessons Learned. Two transconjugants, *Cupriavidus* sp. KN1-TACV and *Cupriavidus* sp. TW2-PV, were cultivated on LB. Both the cultures showed concentrated violet color. The strain KN1-TACV and the strain TW2-PV contained violacein of 4.08±0.05 and 2.74±0.11 fg/cell, respectively. The bacterial cultures were washed and resuspended in a medium containing mineral salts at the concentration of 2 x 10^8 bacterial cells/mL. Then the *Spumella* was added at four different concentrations, 5, 50, 500 and 5000 protozoan cells/mL. The strain KN1-TACV was not predated at all by the *Spumella* under these conditions. On the other hand, the strain TW2-PV inhibit the predation at only 3 lower concentrations of the *Spumella*. It was slowly predated at the initial concentration of 5000 protozoan cells/mL. TCE degradation rates were also examined. The strain KN1-TAC (no violacein producing strain) and the strain KN1-TACV degraded TCE at the rates of 8.18±0.35×10^-10 and 8.91±0.52×10^-10 mg-TCE/(L·hr·cell), respectively. The volacein production did not inhibit TCE degradation at all. The same kind of results were obtained on the strain TW2-PV. Our research showed that the violacein production in bacterial cell is a very powerful tool to protect them from the protozoan predation without no negative effect on the degradability.