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IDENTIFICATION BY STABLE-ISOTOPE PROBING OF MICROBIAL SPECIES
UTILIZING VINYL CHLORIDE AS THE SOLE ENERGY AND CARBON SOURCE

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The use of reductive dechlorinating processes at chloroethene-contaminated sites is widely used to reduce contaminant concentrations below EPA mandated levels. However, one of the major drawbacks to this approach is the accumulation of the recalcitrant intermediate vinyl chloride (VC), a proven human carcinogen. In a previous study, an anaerobic VC enriched consortium consisting of *Flexibacter*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Chlorobium* (Green Sulfur Bacteria) was shown to utilize vinyl chloride as a sole carbon and energy source, producing carbon dioxide as an end product. However, it is unclear which species of bacteria are actively consuming the VC and which were living off of secondary metabolic products. This type of problem is one of the major limitations in using culture-independent molecular techniques in microbial ecology, which, though allowing for identification of previously uncharacterized species, sheds little light on the specific physiologies associated with these phylogenies. The stable-isotope probing (SIP) technique allows for the isolation of DNA associated with specific metabolic activity, such as the utilization of VC as a carbon source. Enrichment cultures were supplied with ^{13}C -labelled VC for a period of approximately two doubling times (~20-30 days) during which several time-point samples were taken for molecular analysis. Extracted genomic DNA was subjected to cesium chloride density gradient equilibrium centrifugation, after which fractions were collected from the ultracentrifugation tube. Fractions were then tested for DNA via PCR amplification of the 16S rRNA gene using the 8F and 1492 eubacterial primers. Those fractions showing positive results underwent digestion using the *HhaI* and *MspI* restriction enzymes and were analyzed using terminal-restriction length fragment polymorphism (T-RFLP). Analysis of this data should allow for the identification of both the species responsible for the primary consumption of vinyl chloride as well as any community shifts associated with the time points. This should provide an initial *in situ* indication of which bacterial species is responsible for the consumption of VC in the enrichment culture. Supported by NIEHS grant number P42-ESO4696