Diversity of 16S rDNA and Naphthalene Dioxygenase Genes from Coal-Tar-Waste-Contaminated Aquifer Waters

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A B S T R A C T

Microbial diversity in four wells along a groundwater flowpath in a coal-tar-waste-contaminated aquifer was examined using RFLP analysis of both 16S rDNA and naphthalene dioxygenase (NDO) genes. Amplified ribosomal DNA restriction analysis (ARDRA) relied upon eubacteria-specific primers to generate four clone libraries. From each library, 100 clones were randomly picked for analysis. Sixty percent of 400 clones contained unique ARDRA patterns. Diversity indices calculated for each community were high (Shannon–Weaver, \( H' = 3.53 \) to 3.69). Clones representing ARDRA patterns found in the highest abundance were sequenced (31 total). Sequences related to aerobic bacteria (e.g., Nitrospira, Methylomonas, and Gallionella) predominated among those retrieved from the uncontaminated area of the site, whereas sequences related to facultatively aerobic and anaerobic bacteria (e.g. Azoarcus, Syntrophus, and Desulfotomaculum) predominated among those retrieved from contaminated areas of the site. Using NDO-specific primers and low-stringency PCR conditions, variability in RFLP patterns was only detected in community-derived DNA (3 of 4 wells) and not in 5 newly isolated naphthalene-degrading pure cultures. The ARDRA patterns of the pure culture isolates were not found in the clone libraries. Polymorphisms in community 16S rDNA and NDO genes found in well-water microorganisms reflected distinctive geochemical conditions across the site. Sequences related to sulfate-reducing bacteria were found in groundwater that contained sulfide, while sequences related to Gallionella, Syntrophus, and nitrate-reducing aromatic hydrocarbon-degrading bacteria were found in groundwater that contained ferrous iron, methane, and naphthalene, respectively.

Introduction

The composition of microbial communities reflects physical, chemical, geological, and biological characteristics of their habitats. Thus, information about key community members can be combined with knowledge of ecosystem
history to develop hypotheses about biogeochemical processes occurring in a given habitat. For example, the composition of bacterial communities present in two deep alkaline, anaerobic aquifers reflected the geochemical signature of the groundwater [16]. Enriched numbers of sulfate-reducing bacteria (SRB) indicated in situ oxidation of organic carbon by SRB where concentrations of sulfate, sulfide, and dissolved inorganic carbon (depleted in \(^{13}\)C) were high [16]. Similarly, in situ reduction of \(^{12}\)CO\(_2\) by autotrophic methanogens was inferred in strata containing high numbers of methanogens, low concentration of dissolved inorganic carbon (DIC), and DIC enriched in \(^{13}\)C [16]. More recently, anaerobic methane oxidation in the marine habitat has been shown to involve a close association of SRB and methane-consuming methanogens by stable isotopic analysis of cell components, fluorescent in situ hybridization, and related sediment characteristics [3, 30, 54]. These examples demonstrate how geochemical and microbialological evidence can be used to document in situ biogeochemical processes.

The paradigm of using convergent lines of geochemical and microbiological evidence to understand biogeochemical processes also applies to in situ metabolism of organic pollutant compounds [4, 20, 31, 43, 51]. At a diesel-fuel-contaminated aquifer, intrinsic bioremediation was documented by isolating \(>10^6\) aerobic and denitrifying petroleum hydrocarbon-degrading organisms per gram; identifying Azoarcus species among the isolates; confirming the abundance of Azoarcus (1% of the community) by hybridization; demonstrating petroleum hydrocarbon degradation concomitant with electron acceptor loss in microcosms; and measuring depleted oxygen and nitrate and higher levels of dissolved inorganic carbon in the contaminated area [31]. Another recent multidisciplinary study of a petroleum–hydrocarbon contaminated aquifer reported that Methanoseta, an acetoclastic methanogen, predominated where methane and DIC concentrations rose [4]. Furthermore, in a BTEX-contaminated aquifer, Geobacteraceae, a family that includes iron-reducing bacterium degraders, predominated in iron-reducing zones [60].

Current molecular methodologies for describing microbial communities (such as amplified ribosomal DNA restriction analysis (ARDRA), DGGE, and t-RFLP) [12, 15, 32, 50, 56] provide an approach to understanding the relationship between habitat geochemistry and native microorganisms. For example, phylogenetic studies of microorganisms in acid mine drainage communities identified novel, uncultured organisms related to the iron-oxidizing groups “Ferroplasma,” Leptospirillum, Sulfolobus, and Acidimicrobium predominant in low-pH, pyrite-rich environments [5, 6]. In addition, at a jet-fuel- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation, ARDRA and sequence analysis demonstrated the presence of a diverse microbial community dominated by Methanoseta and Syntrophus species, indicating that acetoclastic methanogenesis was the final step in hydrocarbon degradation at this site [11]. Bakermans et al. [1] presented data describing geochemical characteristics of a coal-tar-waste-contaminated study site. In this parallel study, we present complementary information on the site’s microbial community. The microbial community in site well waters was characterized by examining (1) naphthalene-degrading pure culture isolates, (2) archetypal naphthalene dioxygenase (naph) genes, and (3) community structure through ARDRA and sequence analysis. Naphthalene dioxygenase sequence diversity was detected only in community extracted DNA, not in pure culture isolates. ARDRA clone libraries were highly diverse and contained a high number of unique sequences for each groundwater sample examined. Differences in the ARDRA-determined community compositions correlated strongly with the coal-tar carbon and energy flow induced by the coal-tar waste.

**Materials and Methods**

**Field Site and Groundwater Sampling**

The site, located in South Glens Falls, NY, has been described previously [43, 49, 66, 69, 72, 73]. Groundwater sampling techniques and locations are described in the accompanying article [1].

**Isolation of Naphthalene-Degrading Bacteria from Groundwater**

On site within 1 h of sampling, a dilution series of groundwater was prepared in phosphate-buffered saline (PBS) (120 mM NaCl, 2.7 mM KCl, 10 mM potassium phosphate buffer pH 7.6) and plated onto Stanier’s minimal salts (MSB) plates [65]. Plates were incubated aerobically with exposure to naphthalene vapors at 10°C [33]. After 4 weeks of incubation, colonies were selected for isolation and were purified by multiple streaking of single colonies on MSB plus naphthalene plates. Naphthalene degradation by isolates was verified by mineralization of \(^{14}\)C-naphthalene [41]. The previously described assay was used with the following modifications. Cells were inoculated in 25-mL screw-cap vials (Pierce, Rockland, IL) containing 3 mL LB (10 g/L tryptone, 5 g/L