Identification of Critical Members in a Sulfidogenic Benzene-Degrading Consortium by DNA Stable Isotope Probing

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Stable isotope probing (SIP) was used to identify the active members in a benzene-degrading sulfidogenic consortium. SIP-terminal restriction fragment length polymorphism analysis indicated that a 270-bp peak incorporated the majority of the 13C label and is a sequence closely related to that of clone SB-21 (GenBank accession no. AF029045). This target may be an important biomarker for anaerobic benzene degradation in the field.

Benzenes is one of the monoaromatic compounds in gasoline (32) and is a carcinogen. Although anaerobic benzene degradation has been studied extensively for the last 2 decades, there is a very limited understanding about the mechanisms of degradation and the organisms that are involved in this process (for reviews, refer to references 4 and 20). Isolates capable of mineralizing benzene under denitrifying conditions have been obtained (5, 11); however, no pure culture capable of benzene degradation under iron-reducing or sulfate-reducing conditions has been identified thus far. The current understanding of anaerobic benzene degradation is based mostly on enrichments, with very little insight into the roles that different microorganisms execute in the mineralization of benzene under anaerobic conditions. In this report, DNA-based stable isotope probing (SIP) and terminal restriction fragment length polymorphism (TRFLP) analysis were used to distinguish the active microorganism(s) in a benzene-degrading sulfidogenic consortium (21). Prior molecular characterization of this original enrichment suggested that the enrichment was made of diverse phylotypes distributed among the classes Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria, the order Cytophagales, and low-G+C gram-positive organisms. Among the 12 bacterial phylotypes identified at that time, 4 were sulfate reducers (22). However, all attempts to obtain a pure culture of a sulfate-reducing benzene degrader have been unsuccessful to date.

To perform the SIP experiment, all cultures were grown in a modified Widdel and Pfennig marine medium (33) at 30°C, with 4.0 g/liter of Na2SO4. A master culture was grown on modified Widdel and Pfennig marine medium (33) at 30°C, between 50 and 500 bp long with a count of more than 50 per unit restriction fragment length polymorphism (TRFLP) analysis was carried out on an ABI 310 genetic analyzer (Foster, CA). TRFs of individual DNA bands were dialyzed (22). However, all attempts to obtain a pure culture of a sulfate-reducing benzene degrader have been unsuccessful to date.

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community fingerprint was also prepared with the genomic DNA from the subcultures.

Gas chromatography-flame ionization detector analysis of the SIP samples showed that nearly half (47 and 57% of $^{12}$C- and $^{13}$C-benzene, respectively) was utilized by day 4 and almost all substrate (87 and 95% of $^{12}$C- and $^{13}$C-benzene, respectively) was utilized within 8 days (Table 1). These data confirm that benzene was degraded by the cultures in the time frame chosen for the SIP experiment.

After DNA was centrifuged for 20 to 24 h, two distinct DNA bands were observed for each gradient under UV transillumination. The $^{12}$C-labeled archaeal carrier DNA, the *E. coli* $^{12}$C$\text{DNA}$, and the bacterial $^{12}$C$\text{DNA}$ formed a separate $^{12}$C$\text{DNA}$ band, while the $^{13}$C$\text{DNA}$ band contained the $^{13}$C-labeled archaeal carrier DNA and any $^{13}$C-labeled bacterial DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium. To test for contamination of the $^{13}$C$\text{DNA}$ band by any $^{12}$C$\text{DNA}$, DNA from the consortium.

Additional evidence supports the notion that phylotype SB-21
with a TRF of 270 bp) is key in the sulfidogenic metabolism of benzene. Primarily, SB-21 has been maintained in this benzene-degrading consortium over more than 10 years of subculturing (22 and this study). Second, increases in the relative peak intensity of the 270-bp TRF corresponds with an almost complete loss of benzene from the active cultures (Table 1). Finally, the 270-bp TRF represents the most prominent peak in the [13C]DNA TRFLP profiles (Fig. 2), indicating that it has incorporated most of the $^{13}$C from $[^{13}$C]benzene into the DNA.

As such, all microorganisms present in this benzene-degrading enrichment could be classified using the following conceptual models: (i) organisms are strictly dependent (i.e., exhibit-
ing syntrophy) (1, 9, 13, 34), showing sequential degradation of metabolites (mutualism) (26), or are fastidious organisms, interdependent for growth factors or nutrients; (ii) organisms have no strict dependence but exhibit coexistence (synergy) (8, 28), possibly feeding off extracellular metabolites of degradation (19); and (iii) all microorganisms degrade benzene but with different efficiencies. Considering that not all the TRFs identified in the master culture were identified in the [13C]DNA (Fig. 2, day 8 to day 15), model iii (all organism are benzene degraders) can be eliminated. Although we could narrow the possible functional models that this consortium is based on, a definitive identification of the relationship between different players needs more extensive investigation. However, we have also tested benzoate, phenol, and toluene (metabolites of benzene degradation [2, 23, 31]) as the sole carbon sources for degradation in this consortium (18). Although benzoate and phenol could be degraded, the rate of degradation was considerably lower and there was a large lag in the onset of degradation, compared to that of benzene-amended cultures. Toluene was not utilized. These results suggest that the labeling of DNA in this SIP experiment, within an 8-day period, is not due to feeding off these metabolites during benzene degradation (mutualism). Even if model i or ii is applied to this consortium, it can be definitively concluded that the phylotype represented by the TRF of 270 bp is crucial to the process of benzene degradation in this consortium, since it is has incorporated the bulk of the carbon from labeled benzene into its DNA, and the change in its relative peak intensity corresponds with the loss of benzene from the culture.

Our findings that a member of the family Desulfobacteraceae plays a key role in benzene degradation is also supported by a recent study (16) in which a dominant phylotype (clone BznS295) in a benzene-degrading marine sulfate-reducing enrichment culture was closely related to SB-21 and SB-30 (Fig. 3). Similarly, research in a column bioaugmented with a methanogenic enrichment (7) showed a correlation between benzene degradation activity and a Desulfobacterium-like clone (OR-M2) (Fig. 3).

In conclusion, these collective results are evidence that SB-21-like organisms are actively involved in benzene degradation in diverse sulfate-reducing and possibly methanogenic environments. Bacteria similar to SB-21 have been identified as one of the dominant microbes in benzene-degrading enrichments established from widely dispersed environments such as a Mediterranean lagoon in France (16) and an oil refinery in Oklahoma (30). Furthermore, the abundance of these 16S rRNA gene sequences could also be linked to benzene degradation (16, 7). Thus, SB-21 potentially could serve as a biomarker for in situ biodegradation of benzene in the environment under sulfidogenic and methanogenic conditions.

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REFERENCES


