

Characterization of the Microbial Communities in Dredge Sediment from a Confined Disposal Facility

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Abstract

Introduction: To maintain the shipping channel at the Toledo Harbor (OH), accumulated sediment is periodically removed by dredging. Material that does not meet quality control standards for contaminant concentrations is deposited into a confined disposal facility (CDF) located near the channel. The presence of contaminants and the continuous management of the CDF creates a unique environmental setting. We hypothesized that bacterial communities in the CDF will exhibit differences in both structure and activity when compared to communities freshly dredged from the shipping channel.

Materials and Methods: Bacterial densities, community level physiological profiling (BIOLOG), an enzymatic assay (fluorescein diacetate hydrolysis, FDA) and molecular techniques (16S rDNA PCR-DGGE and clone libraries development) were used to characterize the microbial communities inhabiting freshly dredged sediment as well as sediments confined in the CDF.

Results: Sediment samples from the CDF exhibited a cell count comparable to the fresh dredge sediments. The average metabolic response (BIOLOG well color development after 156 h) was 1.35 ± 0.04 O.D. in the CDF sediments vs. 1.1 ± 0.03 O.D in the fresh dredge sediments. A greater number of carbon substrates were utilized by communities in the CDF vs. the fresh dredge sediments. The FDA hydrolysis assay revealed significantly greater activity in the shipping channel communities than in the CDF. DGGE analyses revealed a clear pattern of succession in confined- vs. freshly dredged sediments. Sequencing of the 16S rRNA gene libraries showed that Chloroflexi, Bacteroidetes, and Proteobacteria were the major bacteria inhabiting the CDF sediment. In comparison with the microbial communities associated with the shipping channel.

Conclusions: We conclude that the CDF harbors a structurally different bacterial community with higher average metabolic response and metabolic diversity. Significantly, the change in the CDF community has been found to follow a succession trend, where the communities in the newly disposed sediments closely resemble that of the shipping channel, and the communities in older CDF sediments are very different from those of the shipping channel's sediment. Several identified bacteria were described in the CDF environment for the first time, of which many are known to harbor contamination remediation potential.

Objectives

1. Assess the impact of sediment dredging on the structure and activity of indigenous (in the CDF and shipping channel) microbial communities.
2. Identify of major bacterial genera inhabiting the CDF and the shipping channel in order to evaluate the site's intrinsic remediation potential.

Materials and Methods

- Heterotrophic bacteria were enumerated by performing plate counts of sediment dilutions on 1/10 x tryptic soy agar.
- Overall microbial activity was assessed using the (i) fluorescein diacetate hydrolysis assay (FDA, Adam and Duncan 2001), and (ii) average metabolic response (AMR) to single carbon substrates in BIOLOG ecoplates.
- The metabolic diversity of the microbial communities was estimated by summing the number of different carbon substrates (n=31) used in BIOLOG ecoplates.
- The bacterial community structure was assessed through PCR-DGGE fingerprinting (37-50% gradient) using universal bacterial primers for V3 region of the 16S rRNA gene (Muyzer et al. 1993) followed by computerized image analysis.
- Identity of bacterial inhabitants of the sediments was performed by sequencing 16S rRNA genes cloned from phylotypes (n=111) determined to be unique via RFLP analysis (*Taq I* and *Hinf I* digest) of 200 cloned near-full length 16S rRNA genes.

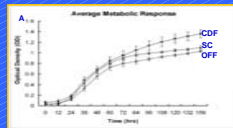


Fig. 1A. Comparison between the metabolic response of the investigated sediment microbial communities. CDF sediment with those from the shipping channel (SC) and offshore (OFF).

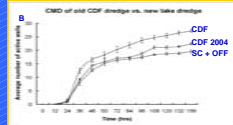


Fig. 1B. Comparing metabolic diversity between older CDF sediment communities, recently deposited sediment (CDF 2004) and shipping channel and offshore communities (SC and OFF respectively).

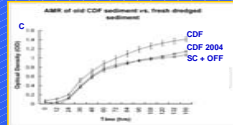


Fig. 1C. Comparing metabolic response between older CDF sediment communities, recently deposited sediment (CDF 2004) and shipping channel (SC) and offshore communities (OFF).

Results

Heterotrophic bacteria enumeration

Bacterial counts of a composted sample of CDF sediment ($5.3 \pm 0.2 \times 10^6$ g⁻¹ dry sediment) were not significantly different than counts of shipping channel sediment ($6.1 \pm 1.4 \times 10^6$ CFU g⁻¹ dry sediment).

Microbial activity

FDA

Microbial communities in the CDF produced 2.32 ± 0.16 µg fluorescein g⁻¹ dry sediment h⁻¹. This value was significantly lower than that observed in shipping channel communities (3.1 ± 0.28 µg fluorescein g⁻¹ dry sediment h⁻¹).

AMR (BIOLOG)

The most recently confined samples (CDF2004) exhibited an AMR that was significantly higher than that of the shipping channel sediments (Fig. 1A and 1B). However, CDF2004 exhibited a lower AMR when compared to older (1980s and 1990s) CDF sediment samples.

Microbial metabolic diversity

The most recently confined samples (CDF2004) exhibited metabolic diversity that was significantly higher than that of the shipping channel sediments (Fig. 1C). However, CDF2004 exhibited a lower metabolic diversity when compared to older (1980s and 1990s) CDF sediment samples.

Bacterial community structure

DGGE

Image analysis of DGGE fingerprints revealed that communities inhabiting the older CDF sediments were more similar to each other than to shipping channel-, offshore disposal site-, and recently deposited (2000s) sediments (Fig. 2). Furthermore, shipping channel-, offshore disposal site-, and recently deposited sediment communities grouped separately from older CDF profiles (Fig. 2).

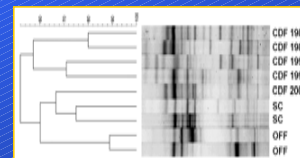


Fig. 2. Dendrogram of DGGE fingerprint similarity. Dates of CDF samples refer to the year of sediment confinement. SC, shipping channel sediment; OFF, offshore disposal site sediment.

Identification of bacteria

Of the 200 clones generated from near-full-length 16S rRNA genes isolated from the CDF and shipping channel, 111 different phylotypes were observed (Fig. 3). Chloroflexi, Bacteroidetes and Proteobacteria bacteria were found to be the most dominant.

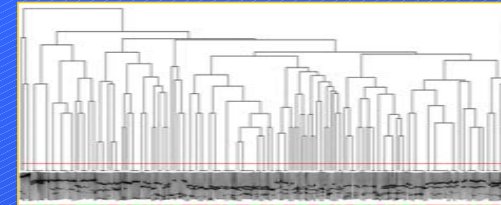


Fig. 3. Dendrogram analysis of full length 16S rDNA gene RFLP. One-hundred and eleven phylotypes were detected assuming a threshold of 90% similarity (red line). Green and red squares indicate RFLP profiles of 16S rRNA genes isolated from the CDF and shipping channel, respectively.

Discussion

- The microbial communities in the CDF exhibited differences in structure and function indicating that a succession of CDF communities is occurring and driving a divergence from the communities found in the habitat of origin (shipping channel).
- The succession of the sediment communities is gradual and time dependent as indicated by trends in AMR, metabolic diversity, and community structure in old vs. recent sediment samples.
- Some of the bacteria identified (*Rhodobium orientis*, *Haliangium tepidum*, *Burkholderia glathei*, *Flavobacterium* spp. *Desulfovibrio* spp. among others) were described for the first time in this environment. Some identified bacteria, such as *Flavobacterium* and *Desulfovibrio*, have been used for remediation of metals. In addition, many isolates appeared to represent novel phylotypes, as no matching BLAST hits were found for their sequences.
- Since the CDF has a history of contamination with metals and some identified CDF bacteria are known to have remediation potential, it can be inferred that the CDF community harbors bacteria that might be useful for intrinsic remediation of contaminated sediments.