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High-Throughput Amplicon Sequencing Reveals Distinct Communities within a Corroding Concrete Sewer System

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Microbially induced concrete corrosion (MICC) is an important problem in sewers. Here, small-subunit (SSU) rRNA gene amplicon pyrosequencing was used to characterize MICC communities. Microbial community composition differed between wall- and ceiling-associated MICC layers. *Acidithiobacillus* spp. were present at low abundances, and the communities were dominated by other sulfur-oxidizing-associated lineages.

Microbially induced concrete corrosion (MICC) is an important problem in sewers. Under anaerobic conditions in wastewater, sulfur-reducing microorganisms convert sulfate and other oxidized sulfur compounds to soluble sulfide and hydrogen sulfide (H₂S) gas (2). Above the sewage level in the pipe, under aerobic conditions, microorganisms associated with concrete surfaces oxidize H₂S to sulfuric acid, which causes concrete corrosion (2, 14). This corrosion results in premature infrastructure degradation or failure, is expensive to repair and mitigate, and presents a potential significant environmental health hazard (14). Consequently, it is critical to improve our understanding of the roles of microbial communities in the corrosion process in order to predict and effectively manage MICC in sewer systems. Recent developments in microbial community analysis techniques have resulted in an opportunity to test the hypothesis that the breadth of microbes associated with concrete sewer pipe corrosion layers is higher than has been previously reported. The finding that organisms other than *Acidithiobacillus thiooxidans* are key community members may also suggest that processes other than those currently known contribute to sewer pipe corrosion.

To date, the majority of MICC studies in sewers have used culture-dependent methods to characterize microbial diversity, and they consistently report that *A. thiooxidans* is the key protagonist (1, 4, 5, 10, 13–15, 17). Three recent studies have used clone libraries of the bacterial 16S rRNA gene to characterize the diversity and composition of microbial communities associated with MICC layers (11, 16, 17). These studies all indicate that while *Acidithiobacillus* spp. can represent major components of some MICC layer communities, they are not ubiquitously dominant. These studies were limited, however, either being based on low sequencing depth (11, 17) or focusing on samples taken from regions, such as manholes (16) or cement coupons in manholes (11), in which conditions are likely to be quite different from those in sewer pipe corrosion layers. Given that culture-dependent methods often poorly represent microbial diversity and that culture-independent methods have focused on samples that may differ from those from sewer pipes, knowledge of the microbial communities associated with MICC layers in sewer pipes needs to be improved.

In this study, the diversity of microbial communities associated with well-established MICC layers in two adjacent but independent sewer pipes receiving the same input wastewater was characterized using universal small-subunit (SSU) rRNA gene

amplicon pyrosequencing (see the supplemental material). We characterized multiple samples with the goal of investigating the variability within these environments and identifying novel microbes associated with MICC.

Ten samples were collected from random positions within the two sewer pipes (see Table S1 and Fig. S1 in the supplemental material). An analysis of environmental monitoring of gas phase temperatures and H₂S levels (data not shown) indicated no statistical difference between the two pipes, with average temperatures of 17.3 to 17.9°C and H₂S levels of 1 to 4 ppm. A minimum of 1,462 amplicon sequences was obtained for each sample, resulting in all data sets being subsampled to a level of 1,400 sequences each for all comparative analyses of diversity. The richness and evenness of microbial communities did not differ between pipes ($P > 0.05$; generalized linear modeling [GLM]) (see Table S1 and Fig. S2 in the supplemental material). Likewise, there was no difference in the composition of microbial communities between pipes ($P > 0.05$; redundancy analysis [RDA]), though principal-component analysis (PCA) revealed that the composition of three samples was distinct from that of the others (Fig. 1). Interestingly, these three samples were taken from MICC layers on the pipe walls, whereas all other samples were collected from sewer pipe ceilings. This difference was significant ($P = 0.013$; RDA) and was related to a greater relative abundance of SSU rRNA gene sequences from an *Acidiphilium* sp. and a *Mycobacterium* sp. in ceiling-associated microbial communities than in wall-associated microbial communities and a greater abundance of *Burkholderiales* spp., *Sphingobacteriales* spp., and *Xanthomonadales* spp. in wall-associated communities than in ceiling-associated microbial communities (Fig. 1 and 2). It is possible that the lower variation in wall samples is the result of occasional inundation during flood events that may act to homogenize the wall environment and limit the development of niche communities. Bacterial populations,

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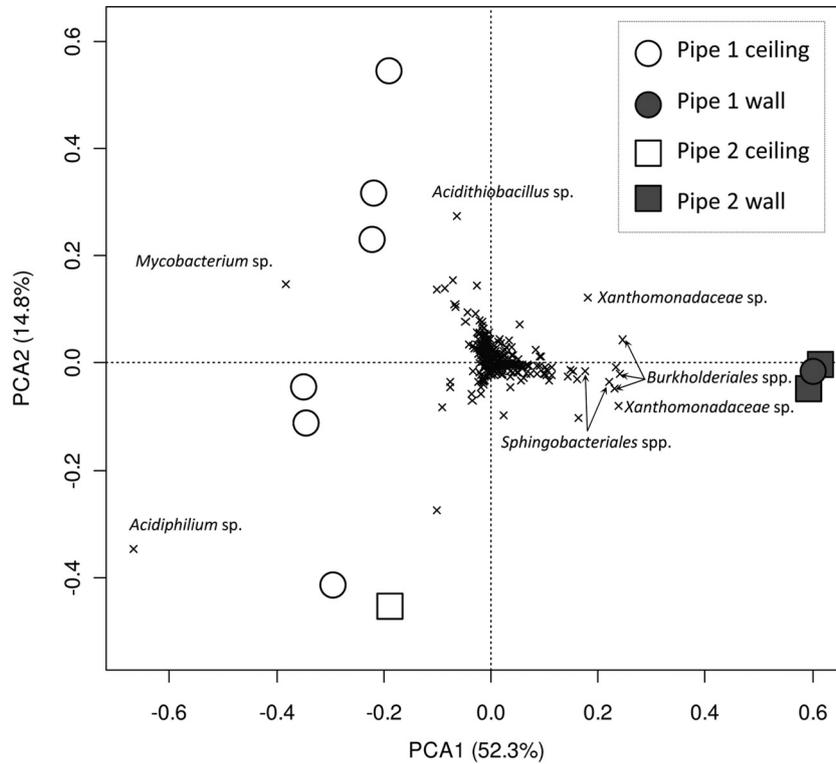


FIG 1 Principal component analysis summarizing variation in the composition of microbial communities associated with MICC layers. Operational taxonomic units are represented by crosses, with the identity of those that discriminate between samples shown in text.

closely related to the abundant species detected here, have been reported, albeit at lower abundances (<3%), in other culture-independent studies of sewer-associated MICC layers (11, 16). Importantly, however, sequences derived from *Acidithiobacillus*

spp. were present at <3% relative abundance in all samples except one (Fig. 2). This finding is in stark contrast with those of the majority of previous studies which indicate that *A. thiooxidans* is the key protagonist of MICC in sewers (10–12, 17).

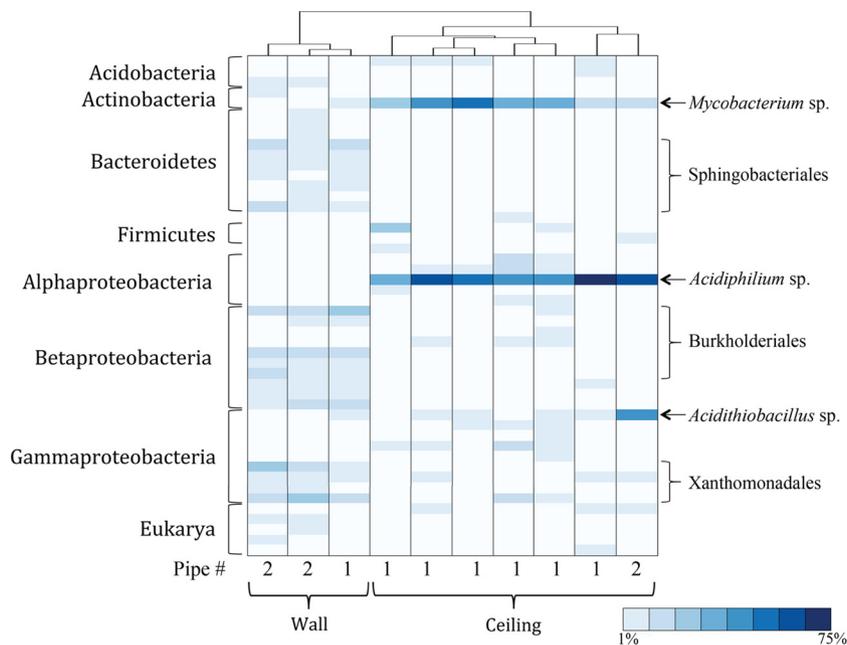


FIG 2 Heatmap summarizing the percent relative abundances of bacteria (each row representing an OTU) that were present at more than 1% in MICC layer samples from walls and ceilings of the two pipes (1 and 2). The relative similarity of each sample in terms of community composition, as determined by complete linkage cluster analysis of OTU abundances, is represented at the top of the heatmap.

The *Acidiphilium* sp. which dominated the ceiling communities was closely related to *Acidiphilium acidophilum*, which is a known sulfur oxidizer (2) and has been previously reported in sewer-associated MICC layers (11). The other dominant population in ceiling-associated MICC layers, a *Mycobacterium* sp., shared 99% nucleotide identity across a 350-bp region of the SSU rRNA gene with a *Mycobacterium* sp. capable of chemolithoautotrophic growth at pH 3.6 by oxidizing sulfur compounds to sulfuric acid (7). The high abundance of these bacteria and their potential sulfur oxidation activities indicate that both the *Acidiphilium* sp. and the *Mycobacterium* sp. are likely protagonists of MICC in sewers. Due to the obligate autotrophic and facultative autotrophic natures of *A. thiooxidans* and *A. acidophilum*, respectively (2), the local level of organic carbon in the corrosion layers likely influenced the relative abundances of at least these organisms.

The relationship between *Burkholderiales* spp., *Sphingobacteriales* spp., and *Xanthomonadales* spp. and MICC in sewers has not been defined. One of the dominant wall-associated *Burkholderiales* sequences showed a high degree of homology to sequences from the genus *Thiomonas* which are known for their ability to oxidize reduced forms of sulfur to sulfate (9). The wall-associated *Xanthomonadales* spp. clustered with a deep-rooted clade of sequences from organisms found in acidic environments, such as acid mine drainage sites (results not shown). This group of *Xanthomonadaceae* spp. appears to be exclusively associated with acidic, sulfur-oxidizing environments and currently has no cultured representatives. The most closely related *Xanthomonadaceae* sequences to this novel group are from the genus *Rhodanobacter*, which includes populations that are capable of sulfur oxidation (8), are abundant in an anaerobic acid environment (3), and have been previously reported in sewer corrosion environments (16). The wall-associated *Sphingobacteriales* spp. are closely related to the *Chitinophagaceae* (6), which are poorly characterized. The presence of these organisms at high abundance in MICC layers suggests that they are contributing to the corrosion process and warrants further investigation.

The use of universal primers has meant that, for the first time in this environment, the relative abundances of lineages from all three domains can be examined. A wide range of eukaryote sequences were present at low abundance, primarily in wall-associated samples. Archaeal sequences were detected only at extremely low levels, with slightly more on the ceiling than on the wall.

This study demonstrates that the compositions of microbial communities associated with MICC layers were similar between these sewer pipes but differed between the pipe walls and ceilings. Given that well-developed MICC layers were present on both the walls and the ceilings, similar corrosion processes are likely to be occurring despite large differences in community composition. *Acidithiobacillus* spp. were generally present at low abundances, which indicated that they were unlikely to be the main protagonists of MICC in these samples. The most likely protagonists of MICC in our study system were *Acidiphilium*, *Mycobacterium*, *Burkholderiales*, *Sphingobacteriales*, and *Xanthomonadales* spp., which were the dominant populations. Many populations closely related to these are capable of oxidizing reduced inorganic sulfur compounds other than H₂S gas; consequently, the focus of corrosion management techniques may need to be modified to account

for these processes. Further culture-independent studies are required to determine whether these populations are dominant in MICC layers in other sewer systems. It is also important to improve understanding of the dynamics of MICC layer-associated community assembly, as this will indicate parameters that can be manipulated to manage MICC in sewers more effectively.

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