Trends in Microbiology

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Review

Microbial Ecology and Evolution in the Acid Mine Drainage Model System

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Acid mine drainage (AMD) is a unique ecological niche for acid- and toxic-metals-adapted microorganisms. These low-complexity systems offer a special opportunity for the ecological and evolutionary analyses of natural microbial assemblages. The last decade has witnessed an unprecedented interest in the study of AMD communities using 16S rRNA high-throughput sequencing and community genomic and postgenomic methodologies, significantly advancing our understanding of microbial diversity, community function, and evolution in acidic environments. This review describes new data on AMD microbial ecology and evolution, especially dynamics of microbial diversity, community functions, and population genomes, and further identifies gaps in our current knowledge that future research, with integrated applications of meta-omics technologies, will fill.

Trends

Contemporary environmental variation determines the distribution patterns of microbial diversity in acid mine drainage.

Relatively deep community genomic sequencing allows genome reconstruction and functional partitioning analysis of both dominant and rare species in microbial communities.

Multi-omics-enabled, community- and organism-wide analyses are revealing the response and adaptation mechanisms of acidophilic microbes to different environmental conditions and stresses.

Evolutionary processes could be inferred from within-population genomic variation.

Acid Mine Drainage as Model Systems

AMD is a worldwide environmental problem primarily resulting from the microbially mediated oxidative dissolution of pyrite (FeS_2) and other sulfide minerals exposed to air and water during mining [1]. Such drainage waters are typically featured by extreme acidity and high concentrations of toxic metals and sulfate, and they represent an extreme environment for life [2]. However, these unique habitats harbor metabolically active, acidophilic microorganisms that are well adapted to the multiple environmental stresses encountered. The importance of AMD environments as model systems for the study of microbial consortia has been well recognized [3,4]. Specifically, the relatively low species richness, limited resource diversity, and tight coupling between biological and geochemical processes of AMD systems make them ideal targets for quantitative, genomic-based analyses of microbial ecology and community function. Moreover, as microorganisms generally occur in AMD as coexisting populations with inherent microdiversity (that is, not clonal populations, which is a typical phenomenon in natural ecosystems [5]), these reduced-complexity systems offer a special opportunity for resolving fine-scale genomic heterogeneity within closely related strains, and the ecological and evolutionary processes inferred may predict patterns in more complex microbial communities [4].

AMD-related microbes thrive in microenvironments, including acid drainage water, sediments, and microbial macroscopic growths [6,7], among which dynamic biotic and abiotic interactions exist. Since the mid-1990s, the community structure and diversity of microbes in a variety of acidic environments in diverse geographical locations have been surveyed by cultivation-independent approaches, including 16S rRNA gene clone libraries and group-specific fluorescence *in situ* hybridization (FISH) [8–11]. More recent studies, in particular those applying high-throughput sequencing technologies, have investigated patterns of, and controls over,

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the ecological distribution of AMD microbes [12–14]. Meanwhile, the development and application of community genomic and postgenomic methods and associated bioinformatics tools have provided unprecedented insights into the genetic and functional dynamics within these extraordinary microbial assemblages [6,15–17]. These have led to increased understanding of community structure, function, and evolution of microbes populating the extreme AMD environment. In this review, we summarize our current knowledge of the microbial ecology of AMD, with a specific focus on patterns of variation in microbial diversity and community functions, and genome dynamics in natural acidophilic populations. Owing to space constraints, we limit our discussion to prokaryotic microbes (bacteria and archaea) that populate typical AMD environments, including acidic, metal-rich mine waters and the streamers and sediments therein, as well as mine tailings actively generating acid drainage.

Distribution Patterns of Microbial Diversity in AMD and Associated Environments

Deciphering the biotic and abiotic factors shaping microbial assemblages over time and along geochemical gradients is a central theme in microbial ecology [18]. While initial molecular inventory studies have expanded our knowledge of the microbial diversity, including detection of less-well-known or novel taxa, of AMD and associated environments [3,19], it is only recently that the spatiotemporal variations in microbial composition and community structure, as well as the primary factors shaping these patterns in these extreme systems, have received considerable attention. Table 1 lists the representative works that adopted an explicit sampling design to explore changes in microbial assemblages along specific gradients of environmental condition. Many of these studies found that pH [20,21], oxidation stage [22], oxygen gradient [23], metal concentration [24,25], and depth [26] were the major environmental determinants of the observed differences in community composition (Table 1). While these surveys have provided initial descriptions of how distinct microbial species may adapt to the different site-specific environmental properties, a general pattern of their ecological distribution remain elusive due to the limited number of samples analyzed and the relatively low resolution offered by the conventional molecular approaches (mainly 16S rRNA clone sequencing). The advent of **high-throughput sequencing** technologies (see Glossary) has allowed sampling microbial diversity more deeply and widely than ever before [27], and thus affords new opportunities for comprehensively examining broader trends of microbial distribution with larger numbers of ecological samples. Using 16S rRNA pyrosequencing, Kuang *et al.* recently examined the biogeographic patterns of microbes from diverse AMD sites across Southeast China (Table 1) [12]. By analyzing 59 natural acidophilic assemblages across a broad range of spatial and physicochemical gradients, this study demonstrated that solution pH is the strongest predictor of community composition in these harsh environments. More importantly, this pH-dependent distribution was identified as a general pattern in a subsequent comprehensive meta-analysis based on molecular inventory studies from globally distributed AMD and associated environments regardless of the long-distance isolation and the distinct substrate types, implying the conservation of ecological niches exhibited by these highly specialized organisms [28]. Indeed, such pH-specific niche partitioning was clearly evident for the dominant lineages such as *Acidithiobacillus*, *Ferrovum*, and *Leptospirillum* [12]. Consistent with this, previous studies have revealed that the lesser known '*Ferrovum*' spp. are more acid-susceptive than the well-studied AMD species *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* and prefer to grow under relatively high ferrous iron concentrations and moderate pH conditions [29,30]. Meanwhile, diverse pH homeostatic mechanisms, including the restrictive proton influx into the cytoplasm with a highly impermeable cell membrane, and the chemiosmotic gradient regulated by the Donnan potential that inhibits proton influx into the cell and pumps excess protons out of the cell [31,32], would explain the predominance of other acidophiles (e.g., *Acidithiobacillus* and *Leptospirillum*) in more acidic environments. Besides, coexisting species may also compete for and occupy different niches

Glossary

Functional partitioning: the partitioning of functions in and among populations within a microbial community.

Genetic recombination: the process of interchanges of chromosomal parts or genes between different microbial strains.

High-throughput sequencing: often refers to a number of different modern sequencing technologies (next-generation sequencing, which is non-Sanger-based) including, for example, Illumina (Solexa) sequencing and Roche 454 sequencing.

Metagenomics: the global analysis of community genomic DNA directly recovered from environmental samples.

Meta-omics: often refers to an emerging field of study in community ecology ending in -omics, such as metagenomics, metatranscriptomics, metaproteomics or metabolomics.

Metaproteomics: the global analysis of proteins expressed by a microbial community.

Metatranscriptomics: the global analysis of transcripts (mRNA) expressed within a microbial community at a given time point.

Microbial biogeography: the study of the distribution of microbial biodiversity over space and time.

Population genomic analysis: the analysis of the form and distribution of genomic variation within natural coexisting microbial populations. This would allow inference of aspects of the evolutionary processes that have shaped the populations.