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Genomic and metagenomic challenges and opportunities for bioleaching: a mini-review

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Abstract

High-throughput genomic technologies are accelerating progress in understanding the diversity of microbial life in many environments. Here we highlight advances in genomics and metagenomics of microorganisms from bioleaching heaps and related acidic mining environments. Bioleaching heaps used for copper recovery provide significant opportunities to study the processes and mechanisms underlying microbial successions and the influence of community composition on ecosystem functioning. Obtaining quantitative and process-level knowledge of these dynamics is pivotal for understanding how microorganisms contribute to the solubilization of copper for industrial recovery. Advances in DNA sequencing technology provide unprecedented opportunities to obtain information about the genomes of bioleaching microorganisms, allowing predictive models of metabolic potential and ecosystem-level interactions to be constructed. These approaches are enabling predictive phenotyping of organisms many of which are recalcitrant to genetic approaches or are unculturable. This mini-review describes current bioleaching genomic and metagenomic projects and addresses the use of genome information to: (i) build metabolic models; (ii) predict microbial interactions; (iii) estimate genetic diversity; and (iv) study microbial evolution. Key challenges and perspectives of bioleaching genomics/metagenomics are addressed.

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1. Introduction

Bioleaching involves the chemical microbe-assisted solubilization of sulfidic minerals for metal recovery at an industrial scale [1–3]. At the present time, copper is the principle primary metal recovered, although other metals such as nickel can be recovered from mixed-metal ores [4]. In general, the heap bioleaching process consists of crushing ore to the size of gravel, piling the crushed ore in a heap and then applying sulfuric acid to the surface of the heap promoting the growth of acidophilic microorganisms (optimal pH for growth <3). The acidophilic microorganisms carry out biochemical

reactions that, coupled with chemical reactions, solubilize the copper [2].

The key aspects of bioleaching that are relevant for this mini-review are that: (i) it involves consortia of acidophilic microorganisms that include chemolithoautotrophic iron and sulfur oxidizers that fix CO₂ from air and heterotrophs that scavenge fixed carbon from the excretions or dead remains of the chemolithoautotrophs; (ii) it involves oxidative processes that use oxygen from the air as a terminal electron acceptor; (iii) several of the key metabolic and chemical reactions involved are exothermic, driving the temperature of the bioleaching heap from ambient temperatures at the beginning to as high as 70 °C over a period of weeks to months [5]; and (iv) the sources of ferrous iron and sulfur compounds that support microbial metabolism are solids, and microbial attack of these substrates often involves cell adhesion and biofilm formation.

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2. Defining the focus of the mini-review

This review focuses on the genomics and metagenomics of acidophilic microorganisms from bioleaching heaps or closely related mining environments. The definition of what constitutes a closely related mining environment is moot. This is an especially important point to clarify because much of the genomic and metagenomic data that has been used to propose models of bioleaching are derived from microorganisms that inhabit other environments such as acid mine drainage (AMD) and acidic (hot) springs. One argument that can be put forward is that many of the microorganisms found in the latter environments are similar, according to 16S rDNA data, to those that have been detected in bioleaching heaps. Therefore, a case can be made that, in the absence of data from authentic bioleaching-derived genomes, related acidophiles serve as credible surrogates for developing genetic and metabolic models of individual species and for suggesting ecophysiological interactions that could occur during bioleaching. But nagging questions arise that need attention; how credible are these models and to what extent can ecophysiological interactions be predicted from such (potentially inadequate or even incorrect) data? Unfortunately, similarity of 16S rDNA sequences between two microorganisms is not sufficient to state that these microorganisms contain the same complement of genes. This concept is developed more fully in a later section (Comparative genomics: estimating genetic and metabolic diversity).

We have included in this mini-review a discussion of genomic and metagenomic information derived from bioleaching heaps and from related mining environments including AMD. We have excluded a discussion of genomics and metagenomics of thick streamer biofilms in AMDs, such as those found in Iron Mountain, because this has been reviewed elsewhere [6,7]. However, we have included some genomes from AMDs that seep out of mines (water-column) and bioleaching heaps. These AMDs share important environmental properties with bioleaching heap environments that help determine microbial composition, such as low pH, high metal concentrations and availability of iron and sulfur. Also, with exceptions such as AMD from coal and lignite mines, both environments are practically depleted of organic matter, a characteristic that promotes the growth of chemolithoautotrophs as drivers of primary biological production [8–10]. However, bioleaching niches are much more variable than AMDs in several of the environmental cues highlighted above, with concentrations of metals and protons building up dramatically during much shorter mineral leaching cycles. Hence, although the microbial biodiversity of bioleaching heaps might be expected to exhibit some similarity to the assemblages of microorganisms from AMD, it displays important differences.

We have also included in this mini-review some genomes from mining-related environments that are not bioleaching heaps. We justify their inclusion because these genomes include the type strains of *Acidithiobacillus ferrooxidans* (coal waste), *Acidithiobacillus caldus* (coal waste) and

Acidithiobacillus thiooxidans (Kimmeridge clay), considered to be major players in bioleaching, and their genomic analyses have produced some of the more advanced models of genetic and metabolic prediction.

3. Current status of genome projects from bioleaching heaps and related mining environments

As of March 2016, there are 157 genomes of acidophiles deposited in public databases. Of these, 29 (20%) are derived from microorganisms associated with bioleaching heaps or related biomining environments (Fig. 1). A list of these genomes is provided in Table 1. Three metagenome studies have been carried out on bioleaching heaps [11,12] (Table 1), whereas ten metagenomic studies of other acidic environments have been published (reviewed in Ref. [13]).

4. Bioinformatic prediction of genetic and metabolic potential

Genomics has allowed unprecedented insights into the genetic and metabolic potential of acidophiles of bioleaching microorganisms and their close relatives. Many of these microorganisms are recalcitrant to genetic manipulation, and bioinformatic analysis of genome information has been a major route for gaining insight into their biology. Bearing the caveat in mind that much of this model building has come from analyses of genomes not directly derived from bioleaching heaps, we address how genome models have been used to predict genes and metabolism and the ecophysiological interactions that are hypothesized to occur during bioleaching.

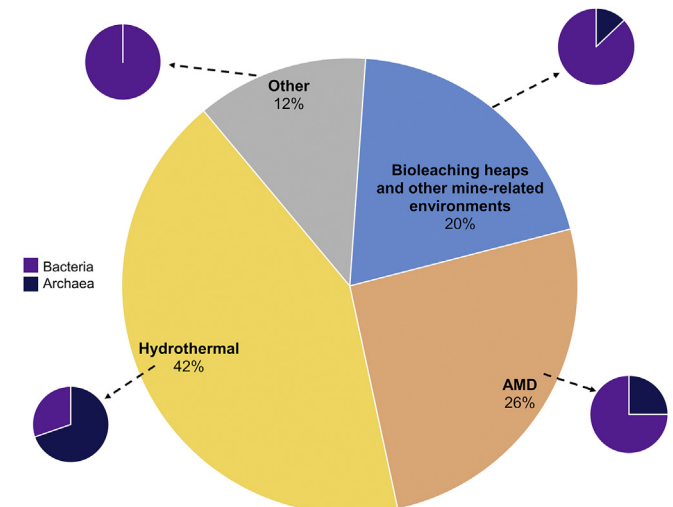


Fig. 1. Distribution of 157 (archaeal and bacterial) acidophile genomes sorted by environmental location: bioleaching heaps and related mining environments, AMD (biofilm streamers), hot springs and other acidic environments. The chart was constructed using information derived from 151 genomes compiled in March 2015 [13] and 6 additional genomes published between March 2015 and March 2016 (NCBI Accession numbers: LPVJ00000000, LJWX00000000, LRRD00000000, LQZA00000000, JFHO00000000, JXYS00000000).

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