Genomics update

Bioleaching genomics

Roland J. Siezen1,2,3* and Greer Wilson4

1Kluyver Centre for Genomics of Industrial Fermentation, TI Food and Nutrition, 6700AN Wageningen, The Netherlands.
2NIZO food research, 6710BA Ede, The Netherlands.
3CMBI, Radboud University Nijmegen, 6500HB Nijmegen, The Netherlands.
4Science Consultant, Bowlespark 30, 6701DS Wageningen, The Netherlands.

Mineral ores are full of metals, some very precious – but how to extract them? The Hamersley mines in the Pilbara in Western Australia contain such rich iron ore that it can almost be welded as it comes out of the ground. Traditionally, metals are extracted by 'smelting' or pyrometallurgy, the thermal treatment of minerals and metallurgical ores and concentrates to bring about physical and chemical transformations, which then enables recovery of valuable metals. But this process is energy-consuming and generates many undesirable side-products such as toxic gases. Alternatively, bioleaching (or biomining) by microorganisms is used to extract metals from ores by dissolving them into extremely acidic aqueous solution. Bioleaching is a natural process involving acidophilic bacteria and archaea, which have the ability to either oxidize metal sulfides or to oxidize reduced inorganic sulfur compounds (RISCs) to sulfuric acid, or both (Fig. 1, left panel). Acid mine drainage liquors were found to contain bacteria responsible for producing iron-rich acidic waters from coal and metal mines. Bioleaching is used today in commercial operations to process ores of copper, nickel, cobalt, zinc and uranium, whereas biooxidation is used in gold processing and coal desulfurization.

The biomining industry has a long-standing interest in the use of extreme acidophiles for metals recovery from ores (for recent reviews see Rawlings, 2002; 2005; Valenzuela et al., 2006; Rawlings and Johnson, 2007). These organisms, with as prime example the mesophilic chemolithotrophic bacterium Acidothiobacillus ferrooxidans, can liberate precious (e.g. gold) and base (e.g. copper) metals trapped in metal sulfides (e.g. iron pyrite and chalcopyrite) through dissimilatory oxidative processes. Biological regeneration of Fe3+ from Fe2+ is the key to chemical attack of metal sulfides. Efficacy in biomining environments also requires tolerance of high levels of toxic heavy metals as well as the ability to assimilate inorganic carbon, as organic sources can be scarce in this environment. A full complement of these desirable traits is not typically present in a single native microorganism, but may be in a consortium. Here we give a brief update of the current status of (meta)genome sequencing and genomics of bioleaching microorganisms and communities.

Fig. 1. (Left) Natural leaching of the mineral chalcopyrite (CuFeS2), which then is precipitated as a mixture of chrysocolla (light blue copper silicate) and malachite (green copper-carbonate-sulfate). The rust-brown colour is a precipitation of iron (oxide/hydroxide), another product of the chalcopyrite leaching reaction. Courtesy of Torbjörn Kjellsson (Kjellsson, 2002). (Right) Large dump-leaching facility at a copper mine operation near Salt Lake City, Utah (http://www.personal.psu.edu/faculty/j/e/jel5/biofilms/leaching.html).

*For correspondence. E-mail r.siezen@cmbi.ru.nl; Tel. (+31) 2436 19559; Fax (+31) 2436 19395.
Chemistry of bioleaching

Metal leaching is mainly a chemical process in which ferric iron and protons are responsible for carrying out the leaching reactions. These reactions take place in the extracellular polysaccharide laid down by cells growing in biofilms rather than cells in a planktonic lifestyle. Biofilm formation greatly accelerates the reactions. There are two types of mechanisms, a thiosulfate mechanism proposed for the oxidation of acid-insoluble metals sulfides, e.g. pyrite (FeS₂) and a polysulfide mechanism for acid-soluble metal sulfides, e.g. sphalerite (ZnS) and chalcopyrite (CuFeS₂). In the thiosulfate mechanism, ferric iron attack solubilizes the acid-insoluble metal sulfide, producing thiosulfate as an intermediate which is then oxidized to sulfate.

\[
\begin{align*}
FeS_2 + 6Fe^{3+} + 3H_2O &\rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+ \\
S_2O_3^{2-} + 8Fe^{2+} + 5H_2O &\rightarrow 2SO_4^{2-} + 8Fe^{3+} + 10H^+
\end{align*}
\]

The role of the microbes is therefore to produce sulfuric acid for proton attack and to keep the iron in the oxidized ferric state for oxidative attack on the metal (Rawlings, 2005). For overviews of acidophilic microorganisms and their chemistry of carbon, iron and sulfur metabolism see Suzuki (2001) and Johnson and Hallberg (2009).

Biomining processes

The engineering options for biomining encompass relatively unsophisticated, inexpensive irrigated dumps (Fig. 1, right panel), to controlled bioleaching from heaps, or to very expensive and highly controlled stirred reactors (Rawlings et al., 2003). The heap reactors now being constructed are stacked, aerated and irrigated. An example of such heaps is in La Escondida, Chile where there are three stacks all around 2–4 km². These are typically used for low-grades ores. These heaps are usually not seeded with any consortia of microbes but are allowed to develop naturally; dormant organisms are present in a quiescent state just waiting for the right conditions. In an industrial setting, bioleaching is started by adding sulfuric acid and aerating the heap. As the leaching progresses, the temperature of the heap increases and so the composition of the microbial communities is constantly changing. The first organisms to act are the mesophilic acidophiles (optimal temperature for growth below 40°C), these are mostly Gram-negative bacteria, next in succession are the moderate thermoacidophiles (40–60°C) which are mostly Gram-positives and finally the extreme thermo-acidiophiles (>60°C), which are mostly Archaea. The latter have been added to heaps from consortia isolated from high-temperature acidic environments. The metabolism of sulfur and carbon in these acidophiles has been reviewed by Johnson and Hallberg (2009). The majority of acidophiles fix carbon dioxide by the Calvin–Benson cycle. The key enzyme here is RUBISCO, and this activity has been detected in these bacteria and confirmed by genome sequencing projects.

Microbial washout from the heap is not a major concern as these microbes tend to grow in biofilms or penetrate into the ore and remain attached. However, the liquor run-off from a heap can be used as the starting culture for a new heap. The liquor is processed by solvent extraction and electro-winning to deposit the metals. Heap leaching takes months rather than years as for dump leaching. Compared with stirred tank reactors, heap reactors form undesired gradients of pH, temperature and reagent levels. Still, the reaction conditions

| Metal leached | Co | Zn/Pb | Au | Cu/Zn/Fe | Cu/Fe |
|---------------|----|-------|----|----------|-------|
| Mineral ore/concentrate | cobaltiferous pyrite | zinc/lead pyrite | (arseno) pyrite | poly-metallic | chalco-pyrite |
| Leaching temperature | 35°C | 35-40°C | 40°C | 45°C | 78°C |
| Species isolated | | | | | |
| Acidithiobacillus caldus | * | * | * | * | * |
| Acidithiobacillus ferrooxidans | * | * | * | * | * |
| Acidithiobacillus thiooxidans | * | * | * | * | * |
| Acidianus sp | | | | | |
| Acidiphilium cryptum | | | | | |
| Leptospirillum ferriphilum | | | | | |
| Metalospheara sp | | | | | |
| Sulfoacidiphilum | | | | | |
| Sulphobacillus thermosulfidooxidans | * | * | * | * | * |

Adapted from Rawlings and Johnson (2007).
Table 2. Genome sequencing projects of microbes involved in oxidation/reduction of iron and/or reduced inorganic sulfur compounds; the majority are acidophiles and are capable of carbon dioxide fixation.

| Kingdom  | Organism                     | Strain   | Phenotype          | Energy source                  | Temperature range | Accession No. | Publication/contact                  |
|----------|------------------------------|----------|--------------------|--------------------------------|-------------------|--------------|-------------------------------------|
| **Complete sequence**                                                                                                     |
| Bacteria | Acidiphilium cryptum         | JF-5     | Iron reducer       | Heterotroph                    | Mesophile         | NC_009484    | microbes@cuba.jgi-psf.org           |
| Bacteria | Acidithiobacillus ferrooxidans | ATCC 53993 | Iron oxidizer   | Obligate, chemolithotroph      | Mesophile         | NC_011206 | borolea@ornl.gov                    |
| Bacteria | Acidithiobacillus ferrooxidans | ATCC 23270 | Iron oxidizer   | Obligate, chemolithotroph      | Mesophile         | NC_011761 | Valdes et al. (2008a)               |
| Bacteria | Leptothrix chalodnii          | SP-6     | Iron oxidizer      | Heterotroph                    | Mesophile         | NC_010524    | demerson@bigelow.org                |
| Archaea | Metallosphaera sedula         | ATCC 51363 | Iron oxidizer   | Chemolithotroph                 | Thermophile       | NC_009440    | Auemik et al. (2008a)               |
| Archaea | Sulfolobus acidocaldarius     | DSM 639  | Sulfur oxidizer    | Lithotroph                      | Thermophile       | NC_007181    | Chen et al. (2005)                  |
| Archaea | Sulfolobus solfataricus       | P2       | Sulfur metabolizing | Lithotroph                      | Hyperthermophile  | NC_002754    | She et al. (2001)                   |
| Archaea | Sulfolobus tokodai            | 7        | Sulfur metabolizing | Lithotroph                      | Hyperthermophile  | NC_003166    | Kawarabayasi et al. (2001)          |
| Bacteria | Sulfitrihydrogenibium azorense | Az-Fu1  | Sulfur oxidizer    | Heterotroph                     | Thermophile       | CP001229     | Reysenbach et al. (2009)            |
| Bacteria | Sulfitrihydrogenibium sp.     | YO3AOP1 | Sulfur oxidizer    | Chemolithoautotroph, Heterotroph | Thermophile       | NC_010730    | Reysenbach et al. (2009)            |
| Bacteria | Sulfiturimona denitrificans   | ATCC 33889 | Sulfur oxidizer, nitrate reducer | Chemolithoautotroph, Heterotroph | Thermophile       | NC_007575    | Sievert et al. (2008)               |
| Bacteria | Thibacillus denitrificans     | ATCC 25259 | Iron oxidizer, sulfur oxidizer | Chemolithoautotroph, Lithotroph | Thermophile       | NC_007404    | Beller et al. (2006)                |
| **Ongoing/draft sequence**                                                                                                  |
| Archaea | Acidianus brierleyi          | JP7      | Iron oxidizer, sulfur metabolizing | Lithotroph                    | Thermophile       | garrett@mermaid.molbio.ku.dk       |
| Bacteria | Acidimicrobiium ferrooxidans  | ICP      | Iron oxidizer      | Autotroph                      | Thermophile       | microbes@cuba.jgi-psf.org           |
| Bacteria | Acidithiobacillus caldus      | ATCC51756 | Sulfur oxidizer   | Chemolithotroph                 | Thermophile       | microbes@cuba.jgi-psf.org           |
| Archaea | Acidithiobacillus thiooxidans | ATCC19377 | Sulfur oxidizer   | Chemolithotroph                 | Mesophile         | microbes@cuba.jgi-psf.org           |
| Archaea | Ferroplasma acidarmanus       | Fer1/Fer1env | Iron oxidizer | Heterotroph                     | Mesophile         | AABC05000000 | Allen et al. (2007)                |
| Archaea | Ferroplasma sp. Type II       | c2       | Sulfur oxidizer    | Chemolithoautotroph             | Mesophile         | AADL00000000 | Tyson et al. (2004)                 |
| Bacteria | Halothiobacillus neapolitanus |          |                   |                                 |                   | microbes@cuba.jgi-psf.org           |
| Bacteria | Leptospirillum ferrooxidans   | DSM 2705 | Iron oxidizer, UBA | Autotroph                      | Mesophile         | microbes@cuba.jgi-psf.org           |
| Bacteria | Leptospirillum sp.            |          |                   |                                 |                   | microbes@cuba.jgi-psf.org           |
| Bacteria | Sulfobacillus acidiphilus     | NAL      | Iron oxidizer, sulfide oxidizer | Autotroph, Mixotroph      | Thermophile       | microbes@cuba.jgi-psf.org           |
| Bacteria | Sulfobacillus thermosulfidoxans | AT-1   | Iron oxidizer, sulfide oxidizer | Autotroph, Mixotroph      | Thermophile       | microbes@cuba.jgi-psf.org           |
| Archaea | Sulfolobus metallicus         |          |                   |                                 |                   | microbes@cuba.jgi-psf.org           |
| Bacteria | Thiomonas intermedia          | K12      | Sulfur oxidizer    | Mixotroph                      | Mesophile         | garrett@mermaid.molbio.ku.dk       |

Adapted from the GOLD Database (http://www.genomesonline.org; February 2009) and CBGB (http://www.cienciavida.cl/CBGB.htm).
are less heterogeneous in a heap than in a dump or in situ leaching operations.

Stirred reactors consist of a series of aerated continuous-flow tanks. These are used for the recovery of high-value metals such as gold. These reactors are expensive to construct but do allow a much more controlled and efficient system for metal recovery. Temperature, pH and aeration are all precisely controlled in order to maintain the desired microbial population. The stirred tanks have many similarities with sewage treatment plants (Siezen and Galardini, 2008). Highly efficient consortia of microbes can be selected and maintained, there is continuous flow, rapid degradation of substrate and less cell washout. Sterility is not essential, as all that is wanted is microbes that can degrade the ore. The consortia of microbes in tanks do change with time, so those that were used to seed the tank will change composition and optimize as the process proceeds. Table 1 lists examples of acidophilic prokaryotes identified in stirred-tank mineral bioleaching and bio-oxidation operations.

Genomics of bioleaching microbes

Table 2 summarizes genome sequencing projects of acidophilic microbes involved in oxidation/reduction of iron and/or RISCs, many of which were isolated from bioleaching operations. Acidithiobacillus ferrooxidans is a major member of microbial consortia used in the bioleaching industry (Table 1). It is abundant in environments associated with pyrite ore bodies, coal deposits and their acidified drainages. Acidithiobacillus ferrooxidans is a chemolithoautotrophic γ-proteobacterium that acquires energy from the oxidation of iron- and sulfur-containing minerals. It is capable of carbon and nitrogen fixation, and thrives at pH of 1–2. The long-awaited, complete annotated genome sequence of the mesoacidophilic A. ferrooxidans ATCC 23270 (3.0 Mb, 58.8% GC) has only recently been published (Valdes et al., 2008a). As expected, the organism was found to have a complete repertoire of genes required for a free-living, chemolithoautotrophic lifestyle, including CO2 fixation, nucleotide and cofactor biosynthesis. Three copies of the gene cluster for RUBISCO were identified, suggesting the ability to adapt to different levels of CO2. Electron transport from iron oxidation is through cytochromes and rusticyanin to cytochrome oxidase and NADH dehydrogenase. The organism can also grow anaerobically and the genome suggests that this is by using sulfur as the final electron acceptor. Figure 2 shows a schematic whole-cell model of functions encoded in the A. ferrooxidans genome (Valdes et al., 2008a), and a first simple metabolic model using flux balance analysis has been reconstructed (Hold et al., 2009). Based on a high-throughput proteomics study of periplasmic proteins, a detailed model was made of location and putative function of many of the periplasmic proteins, including those involved in iron and sulfur oxidation (Chi et al., 2007).
In the complete genome sequence of the extremely thermoacidophilic archaeon *Metallosphaera sedula* (2.2 Mb, 46% GC), genes were identified for iron and sulfur oxidation, autotrophic carbon fixation, metal tolerance and adhesion (Auernik et al., 2008a). Comparative genomics with *A. ferrioxidans* showed that *M. sedula* has different respiratory electron transport chain components, as it does not appear to contain cytochromes. Many of the predicted electron chain components, and several new ones, were identified by global transcriptional analysis of *M. sedula* growing in the presence of ferrous iron and RISCs (Auernik and Kelly, 2008; Auernik et al., 2008a).

A classical metagenome sequencing study of a low-complexity, acid-mine drainage microbial biofilm, growing within a pyrite ore body, allowed the reconstruction of near-complete genomes of the iron oxidizers *Leptospirillum* group II and *Ferroplasma* type II (Tyson et al., 2004). A genome dynamics study in a natural population of the acidophilic archaeon *Ferroplasma acidarmanus*, sampled with a 5-year interval from the same acidic mine site, suggested that gene sequence variability was due to frequent recombination, resulting in a mosaic genome pool (Allen et al., 2007). An oligo-nucleotide microarray has been developed that monitors prokaryotic diversity in extremely acidic environments, including members of the *Nitrospira* phylum, *Acidithiobacillus* genus, acidobacteria, sulfur-reducing bacteria, *Actinobacteria* and *Archaea* of the *Ferroplasma* and *Thermoplasma* genera (Garrido et al., 2008).

### Comparative genomics

Most recently, comparative genomics studies of bioleaching microbes have identified shared or unique adaptation mechanisms. Comparison of genomes of three *Acidithiobacilli* (*A. ferrooxidans, A. thiooxidans* and *A. caldus*) has led to metabolic and regulatory models for each species of electron transfer pathways, *CO₂* fixation, TCA cycle, sulfur oxidation/reduction, iron oxidation, iron assimilation, quorum sensing, hydrogen oxidation, flagella formation, chemotaxis and nitrogen fixation (Valdes et al., 2008b). Predicted interplay between microbes pinpoints possible coordinated responses characteristic of autotrophic microorganisms to environmental signals, such as energy source, oxygen and nutrient limitations, and provides some understanding of how these microorganisms survive and proliferate in extreme environments, including industrial bioleaching operations.

For instance, how do aerobic acidophiles, especially Fe(II)-oxidizers, contend with the paradoxical hazards of iron overload and iron deficiency, each with deleterious

---

© 2009 The Authors
Journal compilation © 2009 Society for Applied Microbiology and Blackwell Publishing Ltd, *Microbial Biotechnology*, 2, 297–303
Future trends, challenges and spin-offs

What is needed in the future for the biomining industry? As bioleaching heaps get hotter they get more efficient, but when the temperature gets too high then the moderate thermophiles becomes necessary. The search is on for microbes which will have a broader temperature range of activity. Will it really be possible to seed bioleaching environments with extreme thermophiles? A biomass of some 15 km² the size of the proposed heap at La Escondida in the near future? New isolates from hot, acidic environments or a GMO organism may help solve these problems. Extreme thermo-acidophile genomes (Auernik et al., 2008b) can be examined for pathways responsible for conferring desirable biomining traits. Taken together with the emerging molecular genetic tools for extreme thermoacidophiles, metabolic engineering of biomining organisms with enhanced properties may soon be a reality. Bioleaching heaps use a tremendous amount of water, so in future much more effort will be spent on ways of remediating the water so that it can be reused in further extractions.

The amount of raw material and accessibility of ores for metal extraction on earth is a finite one. Electronic waste is often full of precious metals and it should also be considered as a potential ‘raw material’ for biomining. Metallurgical processes using chemical leaching of metals are now commonly used, but bioleaching along with biosorption are now being considered to concentrate the leached metals (Cui and Zhang, 2008).

Is there life on Mars? Or has there ever been life on Mars or anywhere else in the universe for that matter? This may seem a strange question to pose at the end of an article on bioleaching/biomining. Astrobiology is making use of the discoveries of bioleaching. Signs Of Life Detector (SOLID2) used a protein chip antibody array to detect microbes, complex molecules and small molecules from mineral deposits (Parro et al., 2008). This work showed that SOLID2 would be capable of detecting similar compounds in samples from space.

Acknowledgements

This project was carried out within the research programme of the Kluiver Centre for Genomics of Industrial Fermentation, which is part of the Netherlands Genomics Initiative/ Netherlands Organization for Scientific Research.

References

Allen, E.E., Tyson, G.W., Whitaker, R.J., Detter, J.C., Richardson, P.M., and Banfield, J.F. (2007) Genome dynamics in a natural archaeal population. Proc Natl Acad Sci USA 104: 1883–1888.

Auernik, K.S., and Kelly, R.M. (2008) Identification of components of electron transport chains in the extremely thermoacidophilic crenarchaeon Metallosphaera sedula through iron and sulfur compound oxidation transcriptomes. Appl Environ Microbiol 74: 7723–7732.

Auernik, K.S., Maaezyato, Y., Blum, P.H., and Kelly, R.M. (2008a) The genome sequence of the metal-mobilizing, extremely thermoacidophilic archaeon Metallosphaera sedula provides insights into bioleaching-associated metabolism. Appl Environ Microbiol 74: 682–692.

Beller, H.R., Chain, P.S., Letain, T.E., Chakicherla, A., Larimer, F.W., Richardson, P.M., et al. (2006) The genome sequence of the obligately chemolithoautotrophic facultatively anaerobic bacterium Thiobacillus denitrificans. J Bacteriol 188: 1473–1488.

Chen, L., Brugger, K., Skovgaard, M., Redder, P., She, Q., Torarinsson, E., et al. (2005) The genome of Sulfolobus acidocaldarius, a model organism of the Crenarchaeota. J Bacteriol 187: 4992–4999.

Chi, A., Valenzuela, L., Beard, S., Mackey, A.J., Shabanowitz, J., Hunt, D.F., and Jerez, C.A. (2007) Periplasmic proteins of the extremophile Acidithiobacillus ferrooxidans: a high throughput proteomics analysis. Mol Cell Proteomics 6: 2239–2251.

Cui, J., and Zhang, L. (2008) Metallurgical recovery of metals from electronic waste: a review. J Hazard Mater 158: 228–256.

Garrido, P., Gonzalez-Toril, E., Garcia-Moyano, A., Moreno-Paz, M., Amils, R., and Parro, V. (2008) An oligonucleotide prokaryotic acidophile microarray: its validation and its use to monitor seasonal variations in extreme acidic environments with total environmental RNA. Environ Microbiol 10: 836–850.

Hold, C., Andrews, B.A., and Asenjo, J.A. (2009) A stoichiometric model of Acidithiobacillus ferrooxidans ATCC 23270 for metabollic flux analysis. Biotechnol Bioeng 102: 1448–1459.

Johnson, D.B., and Hallberg, K.B. (2009) Carbon, iron and sulfur metabolism in acidophilic micro-organisms. In Prin-
ciples of the Magnetic Methods in Geophysics. Poole, R.K. (ed.). Academic Press, pp. 201–255. Kawarabayasi, Y., Hino, Y., Horikawa, H., Jin-no, K., Takahashi, M., Sekine, M., et al. (2001) Complete genome sequence of an aerobic thermoacidophilic crenarchaeon, Sulfolobus tokodaii strain7. DNA Res 8: 123–140. Kjellsson, T. (2002) Chalcopyrite Leaching Followed by Precipitation of Other Minerals [WWW document]. URL http://wiki.biomine.skelleftea.se/wiki/index.php/Chalcopyrite_leaching_followed_by_precipitation_of_other_minerals Osorio, H., Martinez, V., Nieto, P.A., Holmes, D.S., and Quatrini, R. (2008a) Microbial iron management mechanisms in extremely acidic environments: comparative genomics evidence for diversity and versatility. BMC Microbiol 8: 203. Osorio, H., Martinez, V., Veloso, F.A., Pedrosoa, I., Valdés, J., Jedlicki, E., et al. (2008b) Iron homeostasis strategies in acidophilic iron oxidizers: studies in Acidithiobacillus and Leptospirillum. Hydrometallurgy 94: 175–179. Parro, V., Fernandez-Calvo, P., Rodriguez Manfredi, J.A., Moreno-Paz, M., Rivas, L.A., Garcia-Villadangos, M., et al. (2008) SOLID2: an antibody array-based life-detector instrument in a Mars Drilling Simulation Experiment (MARTE). Astrobiology 8: 987–999. Rawlings, D.E. (2002) Heavy metal mining using microbes. Annu Rev Microbiol 56: 65–91. Rawlings, D.E. (2005) Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. Microb Cell Fact 4: 13. Rawlings, D.E., and Johnson, D.B. (2007) The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia. Microbiology 153: 315–324. Rawlings, D.E., Dew, D., and du Plessis, C. (2003) Biomineralization of metal-containing ores and concentrates. Trends Biotechnol 21: 38–44. Reysenbach, A.L., Hamamura, N., Podar, M., Griffiths, E., Ferreira, S., Hochstein, R., et al. (2009) Complete and draft genome sequences of six members of the Aquificales. J Bacteriol 191: 1992–1993. She, Q., Singh, R.K., Confalonieri, F., Zivanovic, Y., Allard, G., Awayez, M.J., et al. (2001) The complete genome of the crenarchaeon Sulfolobus solfataricus. Proc Natl Acad Sci USA 98: 7835–7840. Sievert, S.M., Scott, K.M., Klotz, M.G., Chain, P.S., Hauser, L.J., Hemp, J., et al. (2008) Genome of the epsilonproteobacterial chemolithoautotroph Sulfurimonas denitrificans. Appl Environ Microbiol 74: 1145–1156. Siezen, R.J., and Galardini, M. (2008) Genomics of biological wastewater treatment. Microbial Biotechnology 1: 333–340. Suzuki, I. (2001) Microbial leaching of metals from sulfide minerals. Biotechnol Adv 19: 119–132. Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., et al. (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428: 37–43. Valdes, J., Pedrosoa, I., Quatrini, R., Dodson, R.J., Tettelin, H., Blake, R., 2nd, et al. (2008a) Acidithiobacillus ferrooxidans metabolism: from genome sequence to industrial applications. BMC Genomics 9: 597. Valdes, J., Pedrosa, I., Quatrini, R., and Holmes, D.S. (2008b) Comparative genome analysis of Acidithiobacillus ferrooxidans, A. thioxidans, and A. caldus: insights into their metabolism and ecophysiology. Hydrometallurgy 94: 180–184. Valenzuela, L., Chi, A., Beard, S., Orell, A., Guillani, N., Shabanowitz, J., et al. (2006) Genomics, metagenomics and proteomics in biomining microorganisms. Biotechnol Adv 24: 197–211.