Aspects of the predicted physiology of *Acidithiobacillus ferrooxidans* deduced from an analysis of its partial genome sequence

Marlen Barreto[a], Raquel Quatrini[a], Susan Bueno[b], Claudia Arriagada[a], Jorge Valdes[a], Simon Silver[c], Eugenia Jedlicki[b], David S. Holmes[a,d,*]

[a] *Laboratory of Bioinformatics and Genome Biology, Faculty of Chemistry and Biology, University of Santiago (USACH), 3363, Avenida Bernardo O'Higgins, Santiago, Chile*
[b] *University of Chile, Santiago, Chile*
[c] *University of Illinois, Chicago, IL, USA*
[d] *Millennium Institute of Fundamental and Applied Biology, Santiago, Chile*

Abstract

Candidate genes have been identified in the almost complete genome sequence of *Acidithiobacillus ferrooxidans* that are potentially involved in several physiological processes of relevance to the application of this microorganism to biohydrometallurgical processes. Candidates for six of the 10 missing genes in the previously described amino acid biosynthesis pathways have been located. Genes potentially involved in hydrogen utilization, conjugation, type IV pilus formation, heavy metal resistance and cation fluxes are described.

Keywords: Genome; Metabolic reconstruction; Metal resistance; Amino acid biosynthesis; Conjugation

1. Introduction

Acidithiobacillus ferrooxidans[1] type strain ATCC23270, formerly called *Thiobacillus ferrooxidans* (Kelly and Wood, 2000), is the first biomining microorganism to have had its genome almost completely sequenced (Selkov et al., 2000), with more than 2700 identified open reading frames (ORFs) in about 3 million base pairs (bp) of genome size. It is now possible to begin a genome-wide search for candidate genes for metabolic pathways and other physiological and genetic characteristics of interest. Gene identification and genome organization analysis allow initial predictions to be made as to whether genes are linked in possible functional units. This information can then be used to reconstruct metabolic pathways "in silico" and to begin to unravel the often complicated and multilevel regulation of cellular functions. Missing enzymes and potential bottlenecks can be identified and central control nodes of regulation can be pinpointed. This information is central for the rational planning of ways to genetically manipulate the organism for biotechnological applications.

* Corresponding author. Tel.: +56-2-681-2575x797; fax: +56-2-681-9036.

E-mail address: dsholmes2000@yahoo.com (D.S. Holmes).

[1] *Acidithiobacillus ferrooxidans* will be abbreviated to At.f in this paper.

Many metabolic pathways and physiological/genetic characteristics of At.f have attracted attention and are worthy of further evaluation by whole genomic analysis. These include pathways and regulatory mechanisms for the oxidation of Fe(II), reduced sulfur compounds, formate and hydrogen. Other aspects of interest include, for example, chemotaxis and two-component sensory transduction, CO_2 fixation, carboxysome formation, sulfur and Fe reduction, the roles of transcription factors and stress related proteins, including acid stress and heat stress and an evaluation of the presence and roles of prophage, transposons and introns in the genome. In this interim report, we focus only on the following: amino acid biosynthesis, hydrogen utilization, conjugation, type IV pilus formation, metal resistance and metal cation membrane fluxes. Among the more than 100 bacterial genomes currently available, that of At.f is unusual in that two centers, Integrated Genomics (Selkov et al., 2000) and TIGR have completed partial "gapped" genome sequences (probably more than 95% of the sequence) from the same strain, but neither source is likely to provide a complete genome in the near future. Therefore, analysis of the preliminary sequences is useful.

2. Methods

Metabolic pathways of interest (e.g. amino acid metabolism) were opened in the Integrated Genetics ERGO web site:http://wit.integratedgenomics/IGwit under the section "View Models". Each pathway has hyperlinked connections that allow one to access genes for each of the EC-designated metabolic reactions. Genes accessed from ERGO, or alternatively downloaded directly from the nonredundant data base at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/), were then used to search the At.f ATCC23270 genome in the TIGR (http://www.tigr.org) and ERGO data bases using TBlastN and BlastP, respectively. When a prospective candidate gene of interest was identified in TIGR or ERGO, its predicted amino acid sequence was used to direct a BlastP search of the nonredundant data at NCBI. Only bidirectional best hits were accepted as evidence for putative assignments (Kyrpides et al., 2000).

3. Results and discussion

3.1. Amino acid biosynthesis

One hundred and forty of the 150 genes encoding enzymes involved in the 114 biochemical reactions required for the biosynthesis of the 20 standard amino acids have already been identified in the At.f genome sequence in the ERGO data bank (Selkov et al., 2000). We have identified candidates for six of the 10 missing assignments in the now more complete genome of At.f in the TIGR data base (which is, however, not yet annotated; data not shown). These are genes that encode the following activities: part of a pyruvate dehydrogenase complex (EC1.8.1.4), an asparagine synthetase (EC 6.3.5.4) and enzymes involved in the biosynthesis of chorismate (EC 2.7.1.71), ornithine (EC 1.2.1.38), tyrosine (EC 1.3.1.43) and proline (EC 1.5.1.2).

3.2. Hydrogen utilization

At.f is capable of fixing nitrogen (Mackintosh, 1978) and contains a standard repertoire of genes necessary for nitrogen fixation (Holmes et al., 2001). Hydrogen is an obligatory by-product of nitrogen fixation, consuming about 27% of the electron flux. Some microorganisms have developed processes for recycling hydrogen, thereby increasing the metabolic efficiency of aerobic diazotrophy (reviewed in Friedrich and Schwartz, 1993). Such organisms characteristically contain a membrane bound (NiFe) hydrogenase dimer consisting of an alpha and beta subunit encoded by *hupS* and *hupL* (H uptake small and large subunits) (Fischer et al., 1996). At.f contains homologs of these genes as well as a full suite of *hypA*, *B*, *C*, *D*, *E*, *F* genes arranged in a probable operon that supply and process Ni for the hydrogenase. We also found the activator *hoxA* of the hup operon. At.f has been reported to be able to grow on hydrogen as an energy source both aerobically (Drobner et al., 1990) and anaerobically by the dissimilatory reduction of Fe(III) (Ohmura et al., 1999), although, in the latter case, it was only demonstrated for a private strain (not generally available) of At.f. We did not detect the diaphorase enzyme in At.f that connects the hydrogenase with the formation of NADH. However, this connection might not be

necessary if proton-driven reverse electron flow at pH 2 can be applied to push electrons slightly uphill from the quinone, where the hydrogenase discharges its electrons, to the NAD reductase.

3.3. Conjugation and type IV pilus formation

Conjugal transfer of plasmid DNA from *Escherichia coli* to At.f has been demonstrated (Peng et al., 1994; Liu et al., 2000) and mobilization-type (mob) functions have been described for At.f plasmids (Rohrer and Rawlings, 1992; Drolet et al., 1990). However, due to the lack of an appropriate assay system, conjugal transfer of DNA between At.f strains or from At.f to other organisms has not been described. Inspection of the At.f genome failed to detect any homologs for the described mob functions. This could be due to the lack of these genes in the At.f strain being sequenced (which is different from that in which the mob functions were described) or because the genome sequence is still incomplete.

However, At.f has homologs of almost all the *trb* and *tra* genes required for conjugal transfer of DNA characteristic of the Ti plasmid of *Agrobacterium tumefaciens* and many of these genes are conserved in an order remarkably similar to that found on the Ti plasmid (Fig. 1). Homologs were also found on *luxI* (*traL*) and *luxR* (*traR*). These are involved both in quorum sensing and the subsequent activation of the diverse set of Ti plasmid transfer genes required for formation of a mating or conjugation bridge, activation of the DNA to be transferred and the transfer of DNA itself. These conjugal and transfer functions form part of a type IV secretion system. The essential gene missing in At.f is *trbH*, involved in mating pair formation, and it occupies a position in an operon between the *trbG* and *trbI* genes in the Ti plasmid. In the equivalent putative operon in At.f, we detected a second copy of a *trbG*-like gene (Fig. 1) that might, perhaps, assume the function of the missing *trbH*. *TrbK* is also missing but this function is apparently nonessential.

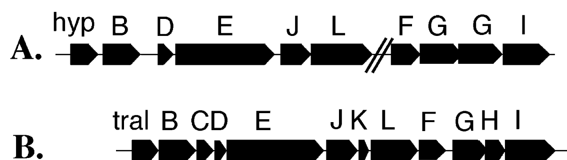

Fig. 1. Genetic map of part of the *tra* region of (A) At.f and (B) the Ti plasmid of *A. tumefaciens*. Arrows indicate the direction of transcription. *hyp* = hypothetical gene that has weak similarity to *traI*. The Ti plasmid region was derived from GenBank 002377 (Suzuki et al., 2000). The double line indicates that the *tra* region of At.f is found on two different contigs in the TIGR database.

In addition, *A. tumefaciens* is capable of conjugally transferring part of its Ti plasmid, termed T-DNA, to a host plant cell. At.f does not have the T-DNA for conjugal transfer of DNA to plants.

At.f has a large repertoire of genes required for pilus formation including *pilA-D*, *pilS* and *pilR*. These genes can be involved in various activities including protein secretion, DNA transfer, adhesion to surfaces and motility (Christie and Vogel, 2000). It also has all the *pil* genes involved in twitching motility that are frequently associated with biofilm formation (Alm and Mattick, 1997). Biofilm formation has been suggested to be important for mineral leaching (Arredondo et al., 1994; Sand et al., 1995).

Especially of interest is the possible role of the *traL* and *traR* genes in quorum sensing in At.f. Quorum sensing responses have been found in a wide variety of gram-negative bacteria and regulate, in addition to conjugation, functions, such as motility, biofilm formation, antibiotic production and the production of virulence factors (Eberl, 1999).

3.4. Heavy metal resistances

Bacteria have genetic determinants of resistances to a large range of toxic heavy metal cations and oxyanions including Ag^{+}, AsO_2^{-}, AsO_4^{3-}, Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Sb^{3+}, TeO_3^{2-}, Tl^{+} and Zn^{2+} (Silver, 1997; Silver and Phung, 1996). Two that have been experimentally determined in At.f are resistances to inorganic mercury (Rawlings and Kusano, 1994) and to arsenic (Butcher et al., 2000), both to As(III) (arsenite) and to As(V) (arsenate). Resistance to Sb(III) has, thus far, always been associated with As(III) resistance where tested but has not yet been evaluated for At.f strain ATCC23270 (Silver and Phung, 1996). Fig. 2 diagrams the genes involved in Hg and As resistance in At.f as published (Inoue et al.,

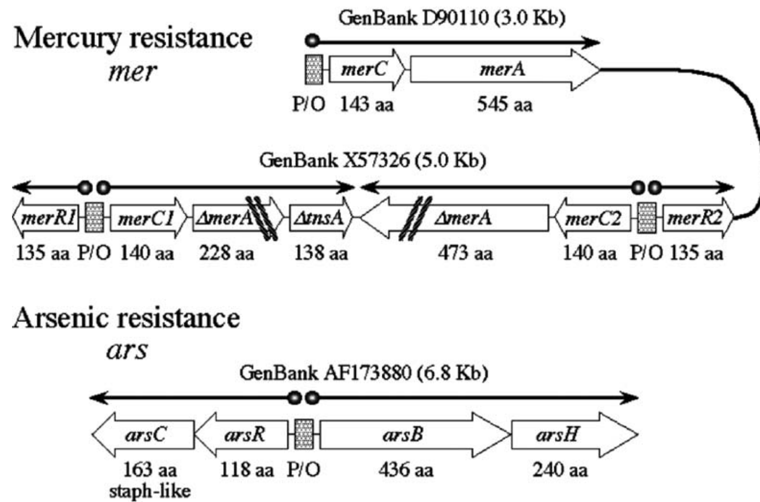

Fig. 2. Genetic map of genes potentially involved in mercury and arsenic resistance in At.f.

1991, 1989 and GenBank AF173880). We have confirmed their occurrence in the TIGR At.f data base.

3.4.1. Mercury resistance

The genetic organization of the chromosomal At.f mercury resistance has been previously determined in a different strain of At.f (Inoue et al., 1989, 1991; Rawlings and Kusano, 1994, GenBank D90110 and X57326). This organization is now confirmed for the sequenced strain. It consists of two unlinked regions, and this organization is unique among the more than 200 *mer* regions analyzed. The first region contains of an operator/promoter region followed by functional *merC* (Kusano et al., 1990) and a *merA* gene (Fig. 2). The more familiar *merT* and *merP* membrane transport genes are missing, and *merC* carries out transport of Hg^{2+} from outside the cell to the intracellular mercuric reductase that is the product of the *merA* gene (Inoue et al., 1996; Kusano et al., 1990; Silver and Phung, 1996). Unlinked to the initial region is a second partial *mer* determinant with two *merC* transport genes, two *merR* regulatory genes and nonfunctional partial *merA* and *tnsA* genes (Fig. 2).

3.4.2. Arsenic resistance

The chromosomal arsenic resistance determinant from still another At.f strain is also somewhat differently organized with regard to genes from other known *ars* determinants (Butcher et al., 2000; Silver and Phung, 1996). It is organized into two divergent operons with the regulatory gene *arsR* and the *arsC* gene determining the enzyme arsenate reductase in one orientation, while the membrane transport gene *arsB*, and a less frequently found gene *arsH* (of unknown function), oriented in the opposite direction (Fig. 2 and GenBank AF173880). The availability of the data now allows for more direct experimentation on arsenic resistance in At.f. By sequence similarity, the ArsC arsenate reductase of At.f is more closely related to the reductase sequence of *S. aureus* (which uses thioredoxin as a redox coupling protein) than to those from *E. coli* and other gram-negative bacteria (which use glutathione and glutaredoxin instead (Silver et al., 2001)). Furthermore, a functional *trxA* (thioredoxin) gene was required for At.f-*arsC*-dependent arsenate resistance in *E. coli,* again indicating a similarity to the *S. aureus* enzyme rather than to the *E. coli* enzyme that requires glutaredoxin but cannot function with thioredoxin (Silver and Phung, 1996). The earlier division of ArsC arsenate reductases into "gram^{+}-like" dependent on thioredoxin and "gram^{-}-like" dependent on glutaredoxin (Silver and Phung, 1996) has recently fallen apart with examples of both types of genes (and presumably enzymes) found in both gram^{+} and gram^{-} bacteria (Silver et al., 2001).

3.4.3. Silver resistance

The genetic/sequence basis for silver resistance in bacteria was initially reported for a plasmid found in *Salmonella typhimurium* (Gupta et al., 1999; Silver et al., 1999, GenBank AF067954). More recently (Gupta et al., 2001), silver resistance *sil* genes have been sequenced from several large plasmids of the IncH incompatibility groups and the chromosomes of several enteric bacteria, including *E. coli*. Genes were found in the At.f genome with significant similarity to several of the silver resistance genes of *S. typhimurium* including *silS*, *R*, *C*, *A* and *P*. However, a critical gene, *silE*, determining a periplasmic silver-binding protein, was not present. We predict that the *sil*-type genes found in At.f are not silver resistance genes, but instead paralogs involved in sensing and efflux of some other (as yet undetermined, but perhaps Cu^{+}) metal cation. A useful point to emphasize is that mechanisms of metal resistance and the genes involved therein frequently appear to be similar in both industrial and clinical microorganisms. Therefore, we can hope that the substantial body of information describing metal resistances in a variety of microorganisms will prove applicable to At.f and other microorganisms involved in bioleaching.

3.5. Metal cation uptake and efflux

Cations, including the major intracellular cations, K^{+} and Mg^{2+}, are major inorganic nutrients for heterotrophic bacteria, as well as chemoautotrophs such as At.f. This topic has been reviewed repeatedly (Saier, 2000; Silver, 1997, 1998; Silver and Walden, 1997). In addition to the "major" nutrient cations, K^{+} and Mg^{2+} and oxyanions PO_4^{3-} and SO_4^{2-}, bacteria often have transport systems for oxyanions of Mo, N (nitrate and/or nitrite) or NH_4^{+}, and for the transport of a wide range of "micronutrient" cations, including those for Co^{2+}, Cu^{+} (or Cu^{2+}), Fe^{2+} (or Fe^{3+}), Ni^{2+} and Zn^{2+}.

Fig. 3 summarizes much of what is known about membrane transport systems limited to divalent cations. Although the information is from a range of other bacteria, the patterns are general and can be applied to the genome data for At.f. First, there are separate uptake and efflux transport systems specific for almost every cation of biological concern. Uptake systems are adapted to nutrient needs, and efflux systems function in maintaining equilibrium and resistance to toxic higher levels of cations in the environment. Starting at about "10 o'clock" in Fig. 3, three transport systems for Mg^{2+} are drawn, as is the case for *E. coli* and *S. typhimurium*. Two are uptake ATPases of the P-type

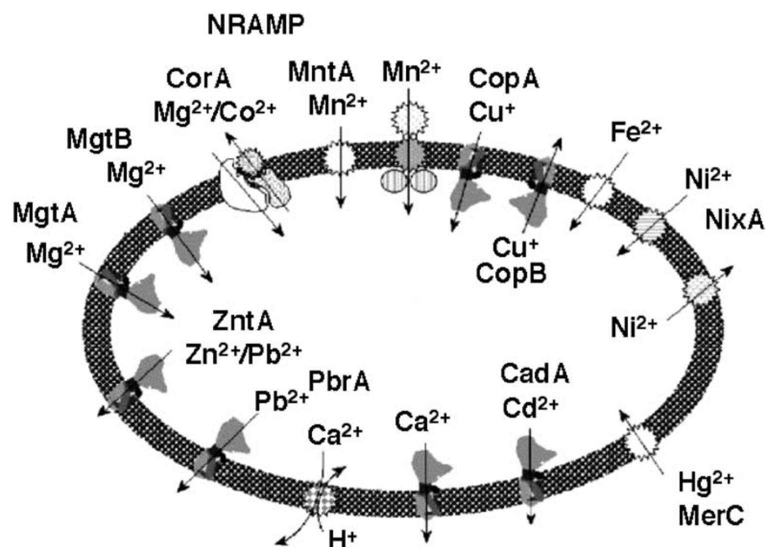

Fig. 3. Diagram of the various influx and efflux mechanisms for divalent cations typically found in bacteria.

class, which are determined by single large genes and contain almost a thousand amino acids (Saier, 2000). They are drawn "duck shaped" with a membrane-embedded section that probably includes 10 transmembrane alpha-helical segments and that includes the cation transport determinant. The head-shaped intracellular domain includes sites for phosphorylation from ATP (hence the name P-type) of a highly conserved aspartate residue, followed by dephosphorylation and removal of the phosphate by a phosphatase domain. The high-resolution crystal structure of a closely related mammalian Ca^{2+} ATPase has recently been solved (Toyoshima et al., 2000). These steps are all parts of the ATPase/cation transport cycle. Both MgtA and MgtB ATPases appear to be unidirectional and involved in uptake of Mg^{2+} only. Mg^{2+} efflux or exchange and efflux of alternative substrates Co^{2+} and Ni^{2+} is carried out by the three polypeptide CorA complex (so named for its role in resistance to high levels of Co^{2+}). CorA functions as a membrane potential-driven cation/cation or cation/H^{+} exchange system and does not involve ATP. Next in Fig. 3 are diagrammed two types of uptake transport systems for Mn^{2+}. A point of interest is the extreme specificity of the Mn^{2+} transporters that discriminate for Mn^{2+} over Mg^{2+} by a ratio of a million or more. The Natural Resistance Associated Macrophage Protein (NRAMP) class of Mn^{2+} transporter also depends on the membrane potential and not on ATP (e.g. Saier, 2000). It is found in virtually all bacterial types, and also in yeast, plants and animals. It is involved in a highly competitive battle over Mn^{2+} by bacteria living alone in external environments or in mixed populations with other microbes, or within plant or animal cells. Those bacterial NRAMP and plant and animal NRAMP proteins are important for plant and animal pathogenesis. While most bacteria have single protein NRAMP Mn^{2+} transporters, some have five polypeptide Mn^{2+} transporters. These are members of a large class of membrane transporters called "ABC" (ATP Binding Cassette) transporters that function sometimes for cations, but at other times (and for heterotrophic microbes) for uptake of amino acids or sugars (Saier, 2000). ABC class transporters have (as shown) an outer membrane protein, a periplasmic substrate binding protein, a pair of inner membrane polypeptides (identical or highly related) and a pair of membrane associated ATPase polypeptides on the inner surface of the inner membrane of gram-negative bacteria. Thus, ABC transporters can consist of up to six polypeptides. The Mn^{2+} transporter is just one group of these.

Continuing around the bacterial cell in Fig. 3, the CopA uptake Cu^{+} P-type ATPase and the CopB efflux Cu^{+} P-type ATPase are diagramed as found in *Enterococcus hirae*. For other bacteria, this is not as well studied. It is also not certain whether Cu^{+} (as found anaerobically and inside the cell) or Cu^{2+} (which is more stable in aerobic environments such as outside of the cell) is the substrate for copper uptake and efflux transporters, which to date appear to be P-type ATPases in several bacterial types. Next, a transporter for Fe^{2+} is diagrammed, although with most bacteria iron transport functions with small iron-specific chelates called siderophores that recognize and bind trivalent Fe^{3+}, the more stable form in aerobic environments (but generally insoluble). If Fe^{3+} is transported, then, it is immediately reduced to Fe^{2+} within the cell, prior to incorporation into heme-containing and other proteins. Many well-known Fe^{3+}-siderophore transporters are ABC-type multicomponent ATPases. Ni^{2+}, which is required by some bacteria for enzymes, such as urease, may be taken up and effluxed (in balanced equilibrium) again by separate transport proteins. Hg^{2+} transport is drawn next in Fig. 3, as the first divalent cation for which there is no "micronutrient" role. Hg^{2+} is purely toxic. As described above, there are, however, transporters for Hg^{2+}, either MerC as found in At.f, or the complex of MerT (a membrane protein) plus MerP (a periplasmic Hg^{2+}-binding protein that is thought to "feed" MerT prior to movement across the membrane). The CadA subfamily of P-type efflux ATPases functions for Cd^{2+}, which is also purely toxic and not a micronutrient. CadA is one of the more widely found and better studied efflux P-type ATPases, so genomic annotations often list "CadA", even if it is likely to transport a divalent cation. Cd^{2+} is generally a high affinity alternative substrate for NRAMP uptake systems, thus necessitating the presence of Cd^{2+} efflux pumps as well.

Ca^{2+} is widely found in bacterial environments, but is generally not used intracellularly and maintained at a very low level within the cellular cytoplasm by highly specific efflux pumps that in different bacteria might be P-type ATPases (Toyosh-

ima et al., 2000) or Ca^{2+}/H^{+} exchange pumps dependent on the membrane potential or pH gradient (as with At.f). Finally, P-type ATPases specific for Pb^{2+} or sharing substrate specificity for Zn^{2+} and Pb^{2+} are shown. Both have been reported in different bacteria with recent experimental support work (e.g. Borremans et al., 2001).

With this wide list of divalent cation uptake and efflux transporters expected to be present in most bacteria, including At.f, we have a starting point for evaluating the first assignments of the At.f genome. The TIGR data set, although better at the moment at the DNA level, has not been annotated, and the list above is quite long, with both a range of orthologs (gene sequences encoding precisely the same gene function in different microbes) and paralogs (encoding related proteins of different substrate specificities, for example P-type ATPases for different divalent cations, frequently in the same organism).

Many attractive candidates are to be found among the 2711 open reading frames listed on the Integrated Genomics At.f site (Selkov et al., 2000 and data not shown) with 1800 tentatively assigned functions. Four open reading frames are annotated as Fe^{3+}-related with two of these close homologs to the *fepC* and *fhuC* genes that determine components of Fe^{3+}-siderophore substrate ABC transporters of *E. coli*. Two are listed as homologs of MntA and MntB that may determine Mn^{2+} transport components in cyanobacteria. Similarly, two open-reading frames in the At.f ERGO data base have been tentatively listed as CopA and CopB (Fig. 3). However, they are most similar to open-reading frames of bacteria that have not been studied for copper transport and only more weakly related to CopA and CopB of *E. hirae*, which is well studied, so their assignment remains very tentative.

A consequence of our bioinformatic analysis has been the identification of a number of candidates for genes determining divalent cation transport and these now await experimental verification.

4. General discussion

Genomics has exploded on the biological world, with numerous examples important to clinical medicine (human and disease-causing pathogen genomes), agriculture (genomes of essential animal and plant product organisms and those of disease-causing microbes), industry (bio-production of ethanol, methane and many small compounds of high-value) and environmental transformations (acidophiles, halophiles and thermophiles). Whole genomic analysis and metabolic reconstruction is particularly powerful for organisms that have not been previously studied, or as in the case of At.f, are recalcitrant to the usual analysis by classical molecular genetics.

The first application of in silico techniques to the genome analysis of At.f was the deduction of the various pathways involved in amino acid biosynthesis (Selkov et al., 2000). This was a logical choice for two reasons. First, At.f is an autotroph and must have the capability to synthesize all of the necessary amino acids. Second, a substantial amount of information exists regarding the genes and enzymes involved in amino acid biosynthesis in many other organisms and this information provides a valuable starting point for searching of similar genes in At.f. Of the 150 genes required for biosynthesis of the standard amino acid, 140 were detected in At.f using the genome sequence in the ERGO data base (Selkov et al., 2000). It was suggested that the 10 missing assignments might not have been detected because the At.f genome in the ERGO data base was incomplete. We have now found candidates for six of the 10 missing assignments in the more complete At.f genome sequence deposited in the TIGR data base, and it is likely that all missing assignments will show up once At.f is completely sequenced and annotated.

In addition to reevaluating amino acid metabolism, we have searched for the presence of genes in At.f that might be involved in several physiological processes of known importance to biohydrometallurgical applications of this microorganism. The physiological processes that we initially set out to investigate also serve as vehicles to illustrate a number of different points regarding the advantages and limitations inherent in bioinformatic interpretations of whole genome sequences.

Certain strains of At.f can grow on hydrogen as an energy source (Drobner et al., 1990; Ohmura et al., 1999). We confirm the presence of genes potentially involved in the oxidation of hydrogen in the sequenced type strain of At.f. This raises the possibility of growing the type strain at nonacidic pHs in which it might be more feasible to promote

conjugation with neutrophilic microorganisms such as *E. coli*.

Whole genome analysis also provides surprises. We went looking for genes related to conjugation because their discovery might aid in designing experiments for DNA transfer to and from At.f. The search was started based on previously published genes related to conjugation and found on At.f plasmids. The result was the discovery of an unrelated set of conjugation-related genes embedded in the chromosome of At.f that displayed remarkable similarity to those found on the Ti plasmid of the plant pathogen *A. tumefaciens*. This raises questions as to the origin of these genes and whether they actually function in At.f for transferring DNA. The Ti plasmid is quite promiscuous and has been found in a number of soil bacteria that normally live at neutral or slightly acidic pH (Teyssier-Cuvelle et al., 1999). How At.f and soil bacteria came to share genes that were probably transferred laterally is a question that merits attention. Finding these genes also raises the question as to how these genes might be exploited for practical applications. Whereas techniques for the conjugal transfer of DNA from *E. coli* into At.f have been developed (Liu et al., 2000), the efficiency of transfer remains low and an alternate, and hopefully more efficient, method for getting DNA into At.f would facilitate its analysis by standard genetic techniques. It would also, in the long term, allow the microorganism to be genetically engineered.

A large number of genes involved in type IV pilus formation have been identified. What is tantalizing is the discovery of a luxIR-type quorum sensing circuit. We speculate that it may be used to control the expression of the putative conjugation genes or perhaps type IV secretion of macromolecules involved in biofilm formation. Hopefully, gene expression analysis using DNA microarrays coupled with molecular biology experiments will help to unravel this interesting problem. DNA expression microarray analysis of At.f is also likely to reveal unexpected and potentially interesting results. For example, three new and unexpected genes, in addition to the *nif* genes, were recently shown by microarray analysis to be regulated by *nifA* (Nienaber et al., 2000) and the discovery that bacterial hemoglobin regulates a suite of unexpected genes has led to its utilization for manipulating fermentation pathways in *Serratia marcescens* (Cameron and Chaplen, 1997; Wei et al., 1998). We expect that DNA microarray analysis and other methods for which the genome is the starting base will also yield important surprises in At.f.

Metal resistance mechanisms and metal ion fluxes clearly play an important role in defining the capacity of At.f to bioleach iron- and copper-bearing minerals especially those that are associated with high concentrations of mercury or arsenic. The organization of genes in At.f involved for mercury resistance has been previously described (Inoue et al., 1989, 1991; Rawlings and Kusano, 1994) and we now confirm this organization in the sequenced strain of At.f and we demonstrate that the more familiar *merP* and *merT* are not found in any other part of the currently available sequence of the genome.

Recently, Velasco et al. (1999) reported a more traditional chromosomally organized *mer* operon, similar to those earlier reported from plasmids and transposons (Silver and Phung, 1996) in a new acidophilic *Thiobacillus* strain T3.2 in which the gene order found was *merR merT merP merA* (GenBank Y11706). No *merC* gene was found. When cloned into *E. coli*, this gene cluster was induced by Hg^{2+} addition. However, the phylogenetic relationship of *Thiobacillus* strain T3.2 to At.f has not been established.

The confirmation of the genetic organization of the genes involved in arsenic resistance in the sequenced strain of At.f and the discovery of genes possibly involved in silver resistance or copper resistance and homeostasis now allow for direct experimentation to investigate the respective mechanism of resistance.

At.f was the first biomining bacterium to have its genome partially sequenced (Selkov et al., 2000). Now, the complete genome sequence of the Fe(II)-oxidizing, acidophilic archaea *Ferroplasma acidarmanus* is available at the DOE Joint Genome Institute web site: http://www.jgi.doe.gov/tempweb/JGI_microbial/html/index.html. The role of *F. acidarmanus* in bioleaching of minerals has not yet been established but it is apparently a dominant organism in mine water at a pH below 1.5 and may be a major contributor to acid mine drainage (Edwards et al., 2000). Probably, other biomining-related microorganisms will be sequenced shortly, which will surely aid in producing a more comprehensive description of how bioleaching

occurs and almost inevitably will lead to improved biotechnological applications of such organisms.

Acknowledgements

We thank Integrated Genomics for making its gapped and partial At.f genome data available. Preliminary sequence data was also obtained from The Institute for Genomic Research website at http://www.tigr.org. Sequencing of *Acidithiobacillus ferrooxidans* was accomplished with support from DOE. We thank L.T. Phung for the preparation of several of the figures. Work supported in part by Fondecyt grant no. 1010623.

References

Alm, R., Mattick, J.S., 1997. Gene 192, 89–98.
Arredondo, R., Garcia, A., Jerez, C., 1994. Appl. Environ. Microbiol. 60, 2846–2851.
Borremans, B., Hobman, J.L., Provoost, A., Brown, N.L., Vander Lelie, D.E., 2001. J. Bacteriol. 183, 5651–5658.
Butcher, B.G., Deane, S.M., Rawlings, D.E., 2000. Appl. Environ. Microbiol. 66, 1826–1833.
Cameron, D.C., Chaplen, F.W., 1997. Curr. Opin. Biotechnol. 8, 175–180.
Christie, P.J., Vogel, J.P., 2000. Trends Microbiol. 8, 354–360.
Drobner, E., Huber, H., Stetter, K.O., 1990. Appl. Environ. Microbiol. 56, 2922–2923.
Drolet, M., Zanga, P., Lau, P.C., 1990. Mol. Microbiol. 4, 1381–1391.
Eberl, L., 1999. Syst. Appl. Microbiol. 22, 493–506.
Edwards, K.J., Bond, P.L., Gihring, T.M., Banfield, J.F., 2000. Science 287, 1796–1799.
Fischer, J., Quentmeier, A., Kostka, S., Kraft, R., Friedrich, C.G., 1996. Arch. Microbiol. 165, 289–296.
Friedrich, B., Schwartz, E., 1993. Annu. Rev. Microbiol. 47, 351–383.
Gupta, A., Matsui, K., Lo, J.F., Silver, S., 1999. Nat. Med. 5, 183–188.
Gupta, A., Phung, L.T., Taylor, D.E., Silver, S., 2001. Microbiology 147, 3393–3402.
Holmes, D.S., Barreto, M., Valdes, J., Dominguez, C., Arriagada, C., Silver, S., Bueno, S., Jedlicki, E., 2001. In: Ciminelli, V., Garcia, O. (Eds.), Biohydrometallurgy: Fundamentals, Technology and Sustainable Development. Elsevier, Amsterdam, pp. 237–251.
Inoue, C., Sugawara, K., Shiratori, T., Kusano, T., Kitagawa, Y., 1989. Gene 84, 47–54.
Inoue, C., Sugawara, K., T. Kusano, T., 1991. Mol. Microbiol. 5, 2707–2718.
Inoue, C., Kusano, T., Silver, S., 1996. Biosci. Biotech. Biochem. 60, 1289–1292.
Kelly, D.P., Wood, A.P., 2000. Int. J. Syst. Evol. Microbiol. 50, 511–516.
Kusano, T., Ji, G., Inoue, C., Silver, S., 1990. J. Bacteriol. 172, 2688–2692.
Kyrpides, N.C., Ouzounis, C.A., Iliopoulos, I., Vonstein, V., Overbeek, R., 2000. Nucleic Acids Res. 28, 4573–4576.
Liu, Z., Guilini, N., Appi-Ayme, C., Borne, F., Ratouchniak, J., Bonnefoy, V., 2000. J. Bacteriol. 182, 2387–2392.
Mackintosh, M.E., 1978. J. Gen. Microbiol. 105, 215–218.
Nienaber, A., Huber, A., Gottfert, M., Hennecke, H., Fischer, H.M., 2000. J. Bacteriol. 182, 1472–1480.
Ohmura, N., Matsumoto, N., Sasaki, K., Nagaoka, T., Saiki, H., 1999. In: Amils, R., Ballester, A. (Eds.), Biohydrometallurgy and the Environment: Toward the Mining of the 21st Century. Elsevier, Amsterdam, pp. 767–775.
Peng, J.B., Yan, W.M., Bao, X.Z., 1994. J. Bacteriol. 176, 2892–2897.
Rawlings, D.E., Kusano, T., 1994. Microbiol. Rev. 58, 39–55.
Rohrer, J., Rawlings, D.E., 1992. J. Bacteriol. 174, 6230–6237.
Saier Jr., M.H., 2000. Microbiol. Mol. Biol. Rev. 64, 354–411.
Sand, W., Gerke, C., Hallman, R., Schippers, A., 1995. Appl. Microbiol. Biotechnol. 43, 961–967.
Selkov, E., Overbeek, R., Kogan, Y., Chu, L., Vonstein, V., Holmes, D., Silver, S., Haselkorn, R., Fonstein, M., 2000. Proc. Natl. Acad. Sci. U. S. A. 97, 3509–3514.
Silver, S., 1997. Rev. Mineral. 35, 345–360.
Silver, S., 1998. J. Ind. Microbiol. 20, 1–12.
Silver, S., Phung, L.T., 1996. Annu. Rev. Microbiol. 50, 753–789.
Silver, S., Walden, W. (Eds.), 1997. Metal Ions in Gene Regulation. Chapman & Hall, New York. 473 pp.
Silver, S., Gupta, A., Matsui, K., Lo, J.-F., 1999. Met-Based Drugs 6, 315–320.
Silver, S., Phung, L.T., Rosen, B.P., 2001. In: Frankenberger Jr., W.T. (Ed.), Environmental Chemistry of Arsenic. Marcel Dekker, New York, pp. 247–272.
Suzuki, K., Hattori, Y., Uraji, M., Ohta, N., Iwata, K., Murata, K., Kato, A., Yoshida, K., 2000. Gene 242, 331–336.
Teyssier-Cuvelle, S., Mougel, C., Nesme, X., 1999. Mol. Ecol. 8, 1273–1284.
Toyoshima, C., Nakasako, M., Nomura, H., Ogawa, H., 2000. Nature 405, 647–656 (and News and Views by D.H. MacLennan and N.M. Green, pp. 633–634).
Velasco, A., Acebo, P., Flores, N., Perera, J., 1999. Extremophiles 3, 35–43.
Wei, M.L., Webster, D.A., Stark, B.C., 1998. Biotechnol. Bioeng. 20, 477–483.