

MDAG.com Internet Case Study 72

Up Close and Personal with an ARD Celebrity: *Acidithiobacillus ferrooxidans*

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(Here is a favourite quotation of mine, also used in MDAG Case Study 36)

“As large animals, we can be forgiven for holding a worldview that celebrates ourselves, but, in truth, this outlook is dead wrong. We have evolved to fit into a bacterial world, and not the reverse... Animals may be evolution’s icing, but bacteria are the cake.” (Knoll, 2003)

Abstract

In many ways, this MDAG Case Study 72 is a continuation of MDAG Case Study 36 from 2010. An important part of Case Study 36 was disparaging *Acidithiobacillus ferrooxidans* for its reputed ability to accelerate pyrite oxidation by one million times. Since then, impressive genomic techniques, refined over the last decade, have revealed much more about *A. ferrooxidans* and other bacteria. This new information further confirms the limits of *A. ferrooxidans* while at the same time showing it can do more than previously thought.

According to the most recent bacterial classification (post-2013), *A. ferrooxidans* is a species in the genus of *Acidithiobacillus* in the Class of *Acidithiobacillia* in the Phylum of *Proteobacteria*. However, “... it is difficult to find a concept that accommodates what microbial taxonomists understand to be a species” and, in 2020, “the structure, ecology, and dynamics of microbial populations are still largely enigmatic, and many aspects of prokaryotic genomics deviate from sexual organisms”.

As a result, the classic concept of species, genus, etc. does not apply reliably to *A. ferrooxidans* and other bacteria. In 2020, a new classification called the “pangenome” was proposed for bacteria and archaea. However, it apparently has not yet been applied to *A. ferrooxidans*.

The pangenome recognizes that species of bacteria cannot be reliably defined because they exchange DNA (like resistance to antibiotics) through several mechanisms grouped as “horizontal gene transfer” or HGT. “This process is so prevalent that DNA can be seen as a public good - a resource that is shared across [bacterial] individuals, populations, and species.” Put simply, anything that one bacterial species has developed to help it thrive over billions of years has probably already been passed on to countless other bacterial species.

Based on “complete genome sequencing” of two strains of *A. ferrooxidans*, many real and inferred genetic-based characteristics are listed in this MDAG Case Study. Many are shared with other bacteria. For example:

- *A. ferrooxidans* can apparently both oxidize and reduce both iron and sulphur for its metabolism and can even live in low-iron environments.
- It has genetic resistance to mercury, arsenate, arsenite, antimony, copper, other metals, aromatic hydrocarbons, and some antibiotics.
- It may be able to precipitate metals from water to the solid phase under anaerobic conditions and also transfer electrons from mineral surfaces to more distant bacteria.
- *A. ferrooxidans* apparently can metabolize or use many elements and compounds, like carbon dioxide, phosphate, nitrogen gas, ammonia, ferric and ferrous iron, sulphide, elemental sulphur, sulphate, formate, and molecular hydrogen.
- Through quorum sensing, *A. ferrooxidans* can communicate with nearby bacteria of many species in order to regulate the ecosystem’s activity.

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1. Introduction

In 2010, MDAG Case Study 36 illustrated persistent, naive, and overly simplistic viewpoints in vogue for bacterial contributions to minesite-drainage chemistry (Morin and Hutt, 2010). One of these viewpoints was that a single species of bacteria could account for most acid rock drainage (ARD), making it an ARD celebrity. This bacterium is now known as *Acidithiobacillus ferrooxidans*.

Case Study 36 provided many reasons why a single bacterial species could not make an enormous contribution under natural conditions in full-scale minesite components. One reason was that a single bacterial species does not exist on its own, but lives in an interacting symbiotic/antagonistic ecosystem with other microbes that typically achieves some type of (meta-)stability.

“Such stability in an ecosystem typically includes predator-prey relationships, where species’ populations are controlled by other species. In turn, this means that any biological effects from one species will be kept under some type of control and stability by others. Put more gently, one species may ‘farm’ another for later food, like our raising cattle or fertilizing soil to increase crop production. We humans could add a phenomenal amount of fertilizer to a hectare of farmland and obtain phenomenal productivity. However, this is not a sustainable process – the soil will degrade and become mostly unproductive.” (Morin and Hutt, 2010)

Global examples of this are the vast and pervasive biogeochemical cycles for sulphur, iron, and carbon (e.g., Wikipedia, 2021h).

Case Study 36's review of bacterial contributions to aqueous chemistry started in the 1920's and eventually:

“... brings us to the wildly distorted ARD story of *Acidithiobacillus*. In particular, one species was sometimes considered the primary cause of pyrite oxidation and ARD. This species was often *A. ferrooxidans*, also known by its older synonyms of *Thiobacillus ferrooxidans*, *Ferrobacillus sulfooxidans*, and *Ferrobacillus ferrooxidans* (U.S. National Center for Biotechnology Information: www.ncbi.nlm.nih.gov).

“Put simply, this bacterium [*A. ferrooxidans*] could reportedly accelerate pyrite oxidation by one million times. Few ever asked questions like:

- ‘It might be able to do that, but like over-fertilizing farmland why would it do that?’
- ‘This bacterium often represents a small portion of the biomass, so what are the other microorganisms doing?’
- ‘If it accelerated the rate that much and the microbial population thus grows exponentially, how are the accumulating waste products removed to prevent [self-]toxicity?’
- ‘What is the evidence that the presence of bacteria is the cause and not the effect of sulphide oxidation?’
- ‘In this biotic world, could we ever expect to see a phenomenally lower abiotic oxidation rate?’”

“During the *Acidithiobacillus* craze in the late 20th century, which seems to be rapidly dying out in the early 21st century, we talked with microbiologists who were frustrated. They knew that *Acidithiobacillus* and similar species comprised only a small percentage of the microbial biomass in minesite components. Also, the identified bacteria and archaea were mostly based on laboratory-based cultures. Many in-field bacteria could not be grown in laboratory-based cultures and thus could not be detected and identified... They warned that the microbial contributions to sulphide oxidation and ARD (acid rock drainage) were thus not well understood, but these warnings were often ignored for decades... Instead, a simple, one-dimensional, one-way, single-species cause-of-an-effect (ARD) was more popular. Now it is worthwhile to return to reality, recalling the previous discussions on predator-prey relationships and microbial ecosystems.” (Morin and Hutt, 2010)

We certainly seriously disparaged *A. ferrooxidans* back in 2010!

However, this is not to say that *A. ferrooxidans* is irrelevant and not important. It makes a contribution like any group in a functioning ecosystem. It is simply that this bacterium is not the overwhelmingly dominant cause of ARD.

Interestingly, with new genomic techniques refined over the last decade, we have learned more about *A. ferrooxidans*. This tells us that *A. ferrooxidans* (1) is probably not a single species as defined for multicellular organisms and (2), through horizontal gene transfer, can cause geochemical effects similar to other unrelated bacteria and those other unrelated bacteria can also do what *A. ferrooxidans* does. These points are the primary topics of this MDAG Case Study 72.

2. Some Biological Details to Start

Biological cells can be divided into two main types: prokaryotic and eukaryotic. A prokaryotic cell has a nucleus that is not enclosed in a nuclear membrane (leading some to say there is no “nucleus”), and a eukaryotic cell has a nucleus encased in a nuclear membrane. Prokaryotes are unicellular and grouped into Bacteria and Archaea. Eukaryotes can be multicellular (like us humans) or unicellular like protozoa, unicellular algae, and unicellular fungi.

The celebrity guest of honour in this MDAG Case Study is the prokaryotic bacterium named *Acidithiobacillus ferrooxidans*. Prior to 2001 and its genetic reclassification, it was known as *Thiobacillus ferrooxidans* and a few other names (see Section 1 above).

Bacteria have been traditionally divided based on their Gram-staining results (Wikipedia, 2021g), with Gram-negative representing most of the bacterial phyla. One phylum of Gram-negative bacteria is *Proteobacteria*, comprised of several classes. Up until 2013, *Acidithiobacillus ferrooxidans* was considered a member of Class *gamma-Proteobacteria*, but then was moved to its own class of *Acidithiobacillia* (Williams and Kelly, 2013).

The number of species within the *Acidithiobacillus* genus may vary with ongoing studies and reclassifications. As of 2020 (Zhang et al., 2020), there were eight validated species: *A. ferrooxidans*, *A. ferriphilus*, *A. ferrivorans*, *A. ferridurans*, *A. albertensis*, *A. thiooxidans*, *A. caldus*, and *A. sulfuriphilus*. The first four obtain energy for growth by using the electron donors of ferrous iron, elemental sulfur, reduced sulfur compounds, hydrogen, or tetrathionate. However, as explained in Section 3 below, “validated species” can have a nebulous and ambiguous meaning for prokaryotes. Also, Section 5 shows that *A. ferrooxidans* can obtain energy from many more sources than those.

Even some species of the genus *Acidithiobacillus* differ significantly, as much as some biological Orders. Williams and Kelly (2013) pointed out:

“It is noteworthy that the genus *Acidithiobacillus* is phylogenetically broad. The *Acidithiobacillus caldus*/*Acidithiobacillus ferrooxidans* intragenus phylogenetic distance is nearly the same (90 %) as that between representatives of the orders *Pasteurellales* and *Enterobacteriales*.

3. Can Bacteria Be Reliably Divided into Species, or is the Pangenome More Realistic?

The previous sections of this MDAG Case Study talked about the bacterial species, *Acidithiobacillus ferrooxidans*. For such a prokaryotic bacterium, the definition of “species” is not as clear or reliable as it is for eukaryotic cells.

In 2001, Rosselló-Mora and Amann (2001) observed:

“Among the microbial taxonomists, there is general agreement that the species concept currently in use is useful, pragmatic and universally applicable within the prokaryotic world. However, this empirically designed concept is not encompassed by any of the, at least, 22 concepts described for eukaryotes.”

“Prokaryotic genomes, in contrast, may undergo homologous recombination with related species that are up to 25% (and possibly more) divergent in the sequences of homologous genes; they can also accept and express new genes on plasmids from extremely divergent sources”

“... it is difficult to find a concept that accommodates what microbial taxonomists understand to be a species.”

“Among the different sources of information that are currently emerging, there is one for which it is difficult to predict how significant it will be for prokaryotic taxonomy: the complete genome sequence... Strain-specific genes, which are assumed to have been acquired by lateral gene transfer, are mostly clustered in single hypervariable regions in what can be considered hot spots of recombination... This might prepare us for a time when whole genome sequencing might be a part of the species circumscription.”

That time has come for *A. ferrooxidans*! The following Section 5 summarizes the relevant findings of its “complete genome sequencing”.

Only just a year ago, Bobay (2020) explained:

“In contrast to animals and plants, where genetic cohesion can essentially be characterized by sexual compatibility and population structure, building a biologically relevant species definition remains a challenging endeavor in prokaryotes. Indeed, the structure, ecology, and dynamics of microbial populations are still largely enigmatic, and many aspects of prokaryotic genomics deviate from sexual organisms.”

To emphasize, even a year ago, “*the structure, ecology, and dynamics of microbial populations are still largely enigmatic*”! The old viewpoints on microbial populations from the 1900's, summarized above in Section 1, now seem even more naive and overly simplistic.

A year ago, Tettelin and Medini (2020) edited an open-access book entitled The Pangenome, where “pangenome” would supercede prokaryotic “species” (see also Costa et al., 2020). Wikipedia (2021a) provided a simple and helpful diagram to explain a pangenome (Figure 3-1). Based on each

“Genome” in Figure 3-1 simplistically representing an individual:

- the Core Pangenome is the set of “gene families” shared by all four individuals (in larger populations, at least shared by 95-100% of all individuals);
- the Shell Pangenome is the set of “gene families” shared by two individuals (in larger populations, shared by 10-95% of all individuals); and
- the Cloud Pangenome is the set of “gene families” in an individual not shared by any other individual (in larger populations, shared by less than 10% of all individuals)

Wikipedia (2021a) reports that the bacterium, *Escherichia coli*, has a genome size of 4000-5000 genes and, based on 2000 genomes (simplistically, individuals), this bacterium has an impressive 89,000 different gene families.

Zhang et al. (2020) reported that genomes for nine strains of *Acidithiobacillus ferrooxidans* are publically available. However, there is no report of an initial attempt of a pangenome for *A. ferrooxidans*. How complex and variable is the bacterial species we call *A. ferrooxidans*? Section 5 below gives us some idea.

The pangenome may be too elegant and elaborate for typical environmental genomics. This is because environmental samples provide an often huge and unsorted collection of genes that are then assigned to various species using human-derived techniques (Wikipedia, 2021b). This is not comparable to a pangenome or complete genome sequencing. However, Ma et al. (2020) explain that habitat-wide “meta-pangenomes” are possible and may help to understand ecosystems.

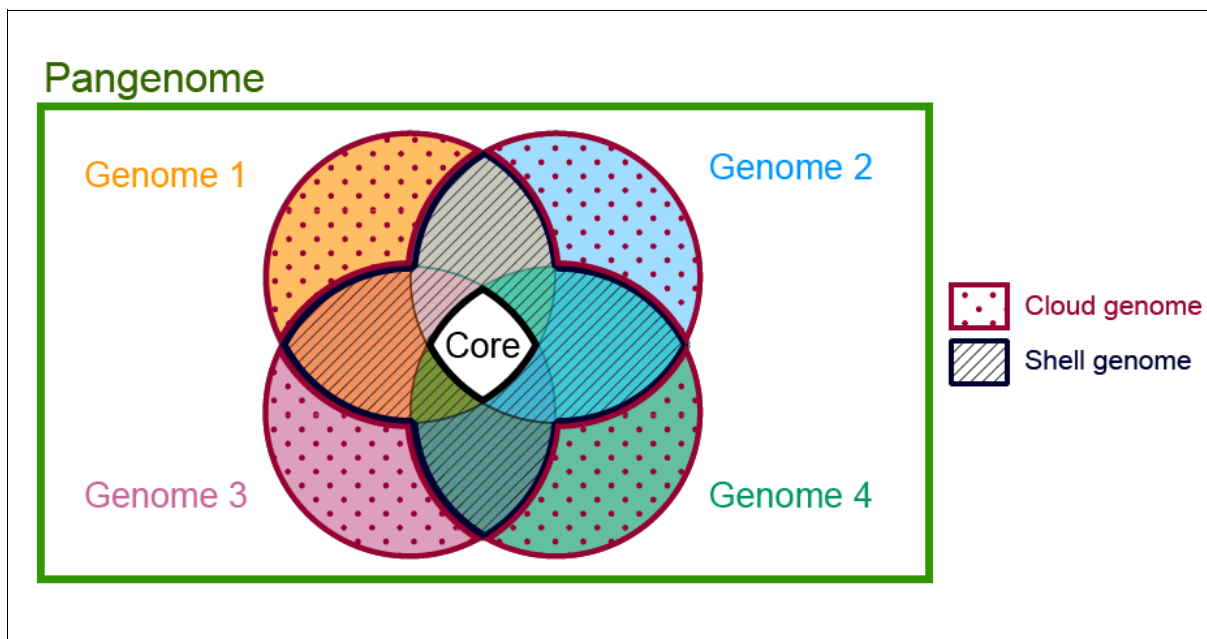


Figure 3-1. Simplified Venn diagram defining core pangenome, shell pangenome, and cloud pangenome (from Wikipedia, 2021a).

4. “Vertical” Reproduction and “Horizontal Gene Transfer” (HGT)

The genetic composition of a bacterial species may be thought to be rigid and well defined. The pangenome (Section 3 and Figure 3-1) casts serious doubt on that. But there is even more ambiguity in the genetic composition of bacterial species - “there’s a lot of gene shuffling going on in bacteria”!

Bacteria and Archaea typically reproduce by fission. This involves the physical division of a cell into two (or more) cells with each having identical DNA. This is often referred to as “vertical reproduction”, because the daughter cells have the same genetic material as the parent cell barring any mutations.

However, although literally not reproduction, bacteria have at least three ways of altering their genetic makeup through “horizontal gene transfer” (HGT).

- 1) transformation: uptake of external genetic material from the surrounding environment (Wikipedia, 2021c)
- 2) transduction: introduction of foreign genetic material into a bacterial cell by a virus (a “bacteriophage”) (Wikipedia, 2021d)
- 3) conjugation: transfer of genetic material between two bacterial cells by direct contact or a physical connection (a bacterial hair-like “pilus”), involving primary “chromosomal DNA” (which exists in the nucleus as a twisted double-stranded loop) and/or secondary DNA molecules (as small double-stranded loops called “plasmids”) outside the nucleus that can replicate (Wikipedia, 2021e)

Wikipedia (2021f) says,

“Horizontal gene transfer (HGT) or lateral gene transfer (LGT) is the movement of genetic material between unicellular and/or multicellular organisms other than by the (‘vertical’) transmission of DNA from parent to offspring (reproduction). HGT is an important factor in the evolution of many organisms.

“Horizontal gene transfer is the primary mechanism for the spread of antibiotic resistance in bacteria, and plays an important role in the evolution of bacteria... It often involves temperate [~infectious] bacteriophages and plasmids. Genes responsible for antibiotic resistance in one species of bacteria can be transferred to another species of bacteria through various mechanisms of HGT such as transformation, transduction and conjugation, subsequently arming the antibiotic resistant genes’ recipient against antibiotics... It is also postulated that HGT promotes the maintenance of a universal life biochemistry and, subsequently, the universality of the genetic code... Most thinking in genetics has focused upon vertical transfer, but the importance of horizontal gene transfer among single-cell organisms is beginning to be acknowledged.”

As a variation on bacterial transduction, alpha-proteobacteria are known to produce virus-like “gene transfer agents” (GTAs), which are different from bacterial transformation, transduction, and conjugation. McDaniel et al. (2010) found that, after two to nine days of incubation in natural

oceanic conditions with GTAs, horizontal gene transfer (HGT) occurred in up to 47% of the abundant culturable natural seawater microbes. They reported that this rate and extent of HGT was one thousand to one hundred million times higher than past estimates. One of the authors reportedly stated, “there’s a lot of gene shuffling going on in bacteria” (Edwards, 2010).

Furthermore, McInerney et al. (2020) viewed HGT as providing a public resource for bacteria:

“The evolution and structure of prokaryotic genomes are largely shaped by horizontal gene transfer. This process is so prevalent that DNA can be seen as a public good - a resource that is shared across individuals, populations, and species. The consequence is a network of DNA sharing across prokaryotic life, whose extent is becoming apparent with increased availability of genomic data.”

With HGT being common and even an integral part of bacterial life, it should not be surprising that *A. ferrooxidans* would contain genes from other species and that other species would contain some *A. ferrooxidans* genes. And this is confirmed in the next section below. It shows that a bacterial species should not be thought of by narrowly defined or limited functions. Put simply, anything that one bacterial species has developed to help it thrive over billions of years has probably already been passed on to countless other bacterial species. That is why Section 3 above asked the question: can bacteria be reliably divided into species?

5. Introducing *Acidithiobacillus ferrooxidans*

As discussed in Section 3 above, “complete genome sequencing” is a recently advanced tool that identifies all the genes, and gene groups or clusters, for a species or strain. Any genes or gene clusters with a known function identified in other species, like oxygen reduction or sulphur oxidation, suggest the subject species may also be capable of this function. It is important to note that the presence of a gene or gene cluster with a known function in other species does not necessarily mean it is active in the subject species.

This section summarizes some findings from the complete genome sequencing of *Acidithiobacillus ferrooxidans* as presented by Valdés et al. (2008), who sequenced Strain ATCC 23270, and Zhang et al. (2020), who sequenced Strain YNTRS-40. Valdés et al. (2008) is much more detailed than Zhang et al. (2020), as seen in the two subsections below. Emphasis here is on genes with potential effects on environmental geochemistry, minesite drainage, and ARD, while genes for other functions like fructose production are not mentioned here.

As explained in Section 3, there is apparently no pangenome yet developed for these and other strains of *A. ferrooxidans*. Thus, the similarities and differences among the strains are not yet clear.

5.1 Strain ATCC 23270

For Strain ATCC 23270 of *A. ferrooxidans*, the following genetic characteristics pertaining to ARD and minesite drainage were observed by Valdés et al. (2008).

- The complete genome size is 2,982,397 base pairs (compared with about 3,200,000,000 base pairs in the human genome) and 3,217 protein-coding genes (compared with at least 20,000 in the human genome).
- 2,070 proteins with known functions were identified.
- *A. ferrooxidans* (in its own class of *Acidithiobacillia* under the phylum of *Proteobacteria*, see Section 2 above) is most similar to the adjacent classes of gamma-proteobacteria (under which it was placed prior to 2013) and beta-proteobacteria. It also shares dozens of protein sequences with Cyanobacteria and Archaea (Archaea are not Bacteria).
- *A. ferrooxidans* can grow by oxidizing ferrous iron and reduced sulphur for aerobic metabolism, and by reducing ferric iron and sulphate for anaerobic metabolism.
- The oxidation of ferrous iron and reduced sulphur compounds (including the oxidation of (1) sulphide to elemental sulphur and (2) tetrathionate $S_2O_4^{6-}$) by *A. ferrooxidans* depends on many enzymes and proteins in the cytoplasmic membrane and between this membrane and the outer membrane (see Figure 3 of Valdés et al., 2008).

- The intake and assimilation of phosphate, organic phosphonate, nitrogen gas, ammonia, ferric and ferrous iron, and sulphate are similarly complicated (see Figure 5 of Valdés et al., 2008). It can also oxidize formate and use molecular hydrogen as an energy source during nitrogen fixation (nitrogen gas to ammonia) or sulphur reduction (elemental sulphur to sulphide).
- Various gene clusters improve survival under variable redox conditions, including fast changing conditions (“stress”), through control of reactive oxygen species (ROS) and of reduced sulphur compounds, repair of oxidative damage, bypassing of damaged functions, ejection of oxidative stress agents, DNA repair, CO₂ fixation, and nitrogen fixation.
- *A. ferrooxidans* may be able to convert carbon to glycogen when sulfur is readily available as an energy source, but use glycogen as a reserve carbon source when iron is readily available as an energy source.
- Because *A. ferrooxidans* thrives in acidic environments, genes include those that may create (1) a proton barrier on the membrane to protect the bacterium, (2) internal ionic gradients with potassium or sodium to inhibit proton influx, and (3) expulsion of protons by enzymes and ion transporters. It can use high aqueous proton activities (low, acidic pH) to drive redox reactions.
- *A. ferrooxidans* can take in ammonia for incorporation into organic compounds such as DNA and amino acids, and can take in sulphate then reduce it to sulphide for cysteine biosynthesis. It can also take in manganese and molybdenum.
- *A. ferrooxidans* “has a surprisingly large number of iron uptake systems” and “its multiple siderophore uptake systems suggest that it is nonetheless capable of living in environments where iron is scarce (perhaps at higher pH values)”.
- Gene clusters provide resistance to mercury, arsenate, arsenite, antimony, copper, and other metals and may be able to precipitate metals from water to the solid phase under anaerobic conditions.
- Gene clusters provide resistance to aromatic hydrocarbons like toluene, xylene, and related aromatic hydrocarbons and to organic solvents. This resistance is suspected to be needed to survive in coal wastes.
- *A. ferrooxidans* may be able to transfer electrons from redox reactions on mineral surfaces to other cells not in contact with the mineral.
- *A. ferrooxidans* is resistant to some antibiotics.
- Several gene clusters are related to the formation of pili (hair-like structures common in bacteria), the adhesion to mineral surfaces, and biofilm formation (autoaggregation and extra-cellular secretions), but not for flagella and chemotaxis (movement) although these have been reported by others.

- Some genes are involved in quorum sensing which ensures a relatively stable ecosystem (e.g., Miller and Bassler, 2001; Wikipedia, 2021i). Through quorum sensing, chemical-signal molecules are released from cells in an ecosystem. This leads to adjustments in individual gene expression, and thus individual metabolism and activity, based on the local density of cells for that species and other species. This may represent a possible evolutionary first step towards multicellular organisms. Quorum sensing in microbial ecosystems within full-scale minesite components would preclude the hyped one-million-times acceleration of pyrite oxidation by *A. ferrooxidans* debunked in Section 1 above.
- A region of the genome shows that *A. ferrooxidans* has been infected by bacteriophages in the past (see transduction in Section 4 above), but no such bacteriophages have yet been identified.
- Despite some contradictory information, *A. ferrooxidans* is likely capable of exchanging DNA with other bacteria through conjugation (see Section 4 above), at least through plasmids. No natural conjugation partners are known, but conjugation between *E. coli* and *A. ferrooxidans* has occurred under laboratory conditions.

5.2 Strain YNTRS-40

For Strain YNTRS-40 of *A. ferrooxidans*, the following genetic characteristics pertaining to ARD and minesite drainage were observed by Zhang et al. (2020). They emphasized that their work characterized the chromosomal DNA in the nucleus separately from DNA in the plasmid.

- The chromosomal DNA is a circular chromosome comprised of 3,209,933 base pairs and the plasmid DNA is a circular chromosome with 47,104 base pairs, for a total of 3,257,037 base pairs (compared with about 3,200,000,000 base pairs in the human genome). There are 3,349 protein-coding genes in chromosomal DNA and 70 in the plasmid DNA (compared with at least 20,000 in the human genome).
- The cells of this strain of *A. ferrooxidans* were rod shaped, with lengths of 1.00-1.68 μm and widths of 0.28-0.40 μm .
- Based on 16S rRNA gene sequencing (not DNA), this *A. ferrooxidans* Strain YNTRS-40 was generally similar to a few other strains of this species.
- 2,015 genes with known functions were identified, plus 1,334 of currently unknown function (Table 6-1).
- Many functional genes allow *A. ferrooxidans* to survive in extreme environments including copper resistance.

Table 6-1. List of functional genes for *Acidithiobacillus ferrooxidans* identified by Zhang et al. (2020)

| <u>Number of Genes</u> | <u>Function in Cluster of Orthologous Groups (COG) Database</u> |
|------------------------|---|
| 1334 | Not in COG database |
| 172 | Translation, ribosomal structure, and biogenesis |
| 162 | Cell wall/membrane/envelope biogenesis |
| 138 | General function prediction only |
| 137 | Amino acid transport and metabolism |
| 135 | Inorganic ion transport and metabolism |
| 129 | Energy production and conversion |
| 126 | Replication, recombination, and repair |
| 118 | Transcription |
| 108 | Coenzyme transport and metabolism |
| 96 | Post-translational modification, protein turnover, chaperones |
| 93 | Carbohydrate transport and metabolism |
| 90 | Defense mechanisms |
| 88 | Mobilome: prophage, transposons |
| 87 | Signal transduction mechanisms |
| 72 | Lipid transport and metabolism |
| 62 | Intracellular trafficking, secretion, and vesicular transport |
| 53 | Nucleotide transport and metabolism |
| 51 | Function unknown |
| 34 | Secondary metabolites biosynthesis, transport, and catabolism |
| 29 | Cell cycle control, Cell division, chromosome partitioning |
| 20 | Cell motility |
| 14 | Extracellular structures |
| 1 | Chromatin structure and dynamics |
| 0 | RNA processing and modification |
| 0 | Nuclear structure |
| 0 | Cytoskeleton |

- Based on Table 6-1, “the functional genes related to defense mechanisms, cell wall/membrane/envelope biogenesis (M), amino acid transport and metabolism (E), inorganic ion transport and metabolism (P) and general function prediction only (R)... revealed that this strain exhibited excellent environmental adaptability...”
- The plasmid DNA was related to secondary metabolism and was believed to assist in handling environmental stress.
- Several coding genes were related to electron transfer.

- Sulphur was the electron donor during early logarithmic growth, then ferrous iron became the electron donor. Sulphur provided more energy (ATP) than ferrous iron per mole.

6. Conclusion

This MDAG Case Study 72 was a continuation of MDAG Case Study 36 from 2010. Since then, impressive genomic techniques, refined over the last decade, have revealed much more about *Acidithiobacillus ferrooxidans* and other bacteria.

One important lesson is that prokaryotic organisms like bacteria cannot be grouped into well-defined species and genera. Over billions of years, they have learned very well how to exchange DNA through various mechanisms of horizontal gene transfer (HGT). HGT renders the concept of distinct species nebulous. In 2020, a new classification called the “pangenome” was proposed for bacteria and archaea. However, it apparently has not yet been applied to *A. ferrooxidans*.

Based on “complete genome sequencing” of two strains of *A. ferrooxidans*, many real and inferred genetic-based characteristics were listed. Many are shared with other bacteria. For example:

- *A. ferrooxidans* can apparently both oxidize and reduce both iron and sulphur for its metabolism and can even live in low-iron environments.
- It has genetic resistance to mercury, arsenate, arsenite, antimony, copper, other metals, aromatic hydrocarbons, and some antibiotics.
- It may be able to precipitate metals from water to the solid phase under anaerobic conditions and also transfer electrons from mineral surfaces to more distant bacteria.
- *A. ferrooxidans* apparently can metabolize or use many elements and compounds, like carbon dioxide, phosphate, nitrogen gas, ammonia, ferric and ferrous iron, sulphide, elemental sulphur, sulphate, formate, and molecular hydrogen.
- Through quorum sensing, *A. ferrooxidans* can communicate with nearby bacteria of many species in order to regulate the ecosystem’s activity.

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