The biofilm lifestyle and cell-cell interactions within acidophilic leaching bacteria

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Biomining uses acidophilic microorganisms for the recovery of metals from sulphide ores in tanks, heaps and dumps. Bioleaching of copper minerals such as chalcopyrite ($CuFeS_2$; the largest copper resource in the word) is done in engineered heaps and accounts for approximately 15% of the present world copper production. Heap bioleaching is a very slow process that can take up to several years to achieve good metal recoveries. In order to enhance metal recoveries, usually the ores are inoculated with a mix of biomining microorganisms before the heap is constructed.

Bacterial cells can effect this metal sulfide dissolution via iron(II) ion and/or sulfur compound oxidation. Biofilm forming cells are the ones which start the leaching process. These are embedded in a matrix of extracellular polymeric substances (EPS), which complex iron(III) ions (Fig. 1). EPS are responsible for an electrostatic mediation of cell attachment to metal sulfides, while their second function is the oxidative dissolution of the metal sulfide[1].

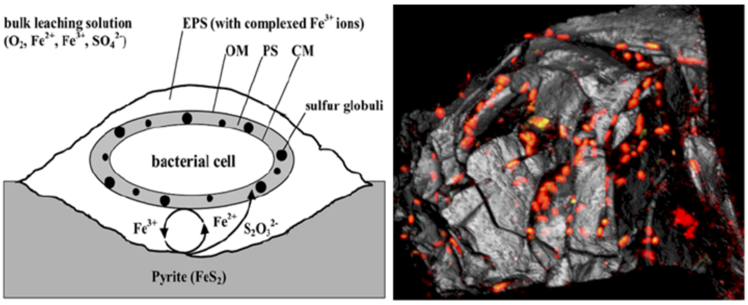

Fig. 1. *Model for contact leaching catalysed by* *<u>At. ferrooxidans</u>* *(left) embedded in its EPS attached to pyrite (CM, cytoplasmic membrane; PS, periplasmic space; and OM, outer membrane). Confocal laser microscopy image showing a 3D projection of a pyrite grain (50-100 µm) colonised with* *<u>At. ferrooxidans</u>* *(the colonisation pattern correlates with surface imperfections). Cells were double stained with syto9 (Green; cell stain) and the lectin ConA (Red; polysaccharide stain)*

Biofilm formation is a genetically regulated process where the microorganisms involved undergo a series of changes in their gene expression resulting in molecular adaptations to their new sessile lifestyle. One of the main phenotypic changes in an intense EPS production[2]. We have studied the molecular adaptations to the biofilm lifestyle in *At. ferrooxidans* by high throughput proteomics. After 24 h contact with the mineral, we detected 1319 proteins were in both planktonic and sessile (biofilm) cell subpopulations. Changes between planktonic cell subpopulations accounted for 16% of the proteins reliable detected. This suggests that the early steps of *At. ferrooxidans* biofilm formation consist of a set of metabolic adaptations after cell attachment to the mineral surface. Functions such as extracellular polymeric substances biosynthesis, responses against oxidative stress seem to be pivotal[3].

Interestingly, the biosynthesis of capsular polysaccharides from EPS is regulated at several levels, including inorganic phosphate starvation or the presence of reduced sulfur compounds[2]. *At. ferrooxidans* possess a functional LuxIR-type I Quorum Sensing system and produce the autoinducers N-acyl homoserine lactores (AHLs)[4]. We positively modulated its biofilm formation on sulfur and pyrite surfaces by the external addition of AHLs with a long acyl chain (12 or 14 carbons). This enhancement correlated with an enhanced capsular polysaccharides[5] (Fig 2).

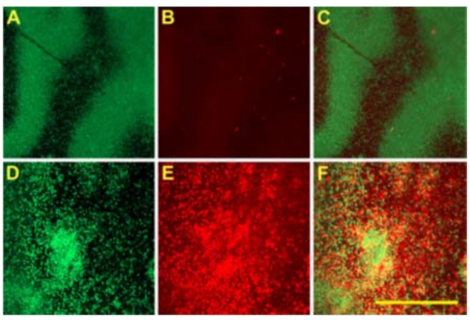

Fig. 2. *At. ferrooxidans* polysaccharides from EPS are induced by the C14- and 3-hydroxy-C14-AHL mixture.
<u>At. ferrooxidans</u> biofilms were grown on floating filters and incubated without agitation at 28 °C. After 6 days of incubation, filters were double stained. The upper panel shows a control filter with no addition of the AHL mixture. The lower panel shows a filter incubated with 0.5 µM hydroxy-C14-AHL and C14-AHL mixture. Confocal Laser Scanning Microscopy images for cell stain Syto9 (a, d), Lectin stain ConA, specific for polysaccharides (b, e), and merged images (c, f) are shown. Size bars represent 20 µm

Cell-to-cell communication among various acidophilic leaching bacteria has not been studied in detail. In our recent work, we focused on the effects exerted by the external addition of mixtures of synthetic AHLs on pure and binary cultures. Results revealed that some mixtures had inhibitory effects on pyrite leaching (Fig. 3) Some of them correlated with changes in biofilm formation patterns on pyrite coupons. We also provide evidence that *At. thiooxidans* and *Acidiferrobacter* spp. produce AHLs and could sense them. In addition, the observation that *At. thiooxidans* cells attached more readily to pyrite pre-colonized by live iron-oxidizing acidophiles than to heat-inactivated or biofilm-free pyrite grains suggests that other interactions also occur[6].

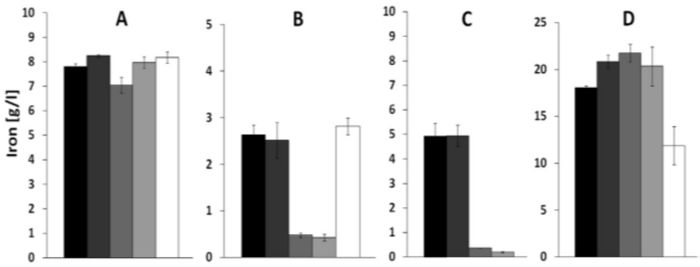

Fig. 3. Influence of AHL-mixture addition on pyrite dissolution in pure cultures. *Pyrite leaching assays in pure cultures of At. ferrooxidans^{T} (A), At. ferrivorans SS3 (B), Acidiferrobacter sp. SPIII/3 (C) and L. ferrooxidans DSM 2391 (D) were incubated at 28 °C with 120 rpm shaking. Total iron concentrations were determined after 28 days of incubation. Mean values from duplicate assays are shown. Black bars correspond to control experiments without addition of AHLs. Colors from left to right correspond to cultures amended with C8-, C12-, C14- and C16/18-AHLm, respectively.*

The understanding of cell-to-cell communication may consequently be used to develop future attempts to influence biomining/bioremediation processes. This include potential natural inhibition of acid mine drainage by enhancing antagonistic interactions among leaching bacterial communities.

References

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