Extremophiles (2010) 14:485–491
DOI 10.1007/s00792-010-0328-1

ORIGINAL PAPER

Biofilm development in the extremely acidophilic archaeon '*Ferroplasma acidarmanus*' Fer1

Craig Baker-Austin · Joanna Potrykus · Margaret Wexler · Philip L. Bond · Mark Dopson

Received: 3 May 2010 / Accepted: 25 August 2010 / Published online: 12 September 2010

Abstract '*Ferroplasma acidarmanus*' Fer1 is an iron-oxidizing extreme acidophile isolated from the Iron Mountain mine, California, USA. This archaeon is predominantly found in biofilm-associated structures in the environment, and produces two distinct biofilm morphologies. Bioinformatic analysis of the '*F. acidarmanus*' Fer1 genome identified genes annotated as involved in attachment and biofilm formation. No putative quorum sensing signaling genes were identified and no *N*-acyl homoserine lactone-like compounds were found in '*F. acidarmanus*' Fer1 biofilm supernatant. Scanning confocal microscopy analysis of biofilm development on the surface of pyrite demonstrated the temporal and spatial development of biofilm growth. Furthermore, two-dimensional polyacrylamide gel electrophoresis was used to examine differential protein expression patterns between biofilm and planktonic populations. Ten up-regulated proteins were identified that included six enzymes associated with anaerobic growth, suggesting that the dominating phenotype in the mature biofilm was associated with anaerobic modes of growth. This report increases our knowledge of the genetic and proteomic basis of biofilm formation in an extreme acidophilic archaeon.

Keywords *Ferroplasma* · Archaea · Acidophile · Biofilm · Confocal microscopy

Abbreviations

EPS	Extracellular polymeric substances
HSLs	*N*-acyl homoserine lactones
MSM	Mineral salts medium
2D-PAGE	2-Dimensional polyacrylamide gel electrophoresis
SAM	*S*-adenosylmethionine
MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight

Communicated by A. Driessen.

Electronic supplementary material The online version of this article (doi:10.1007/s00792-010-0328-1) contains supplementary material, which is available to authorized users.

C. Baker-Austin · M. Wexler · P. L. Bond · M. Dopson
School of Biological Sciences,
University of East Anglia,
Norwich NR4 7TJ, UK

J. Potrykus · M. Dopson (✉)
Molecular Biology, Umeå University,
901 87 Umeå, Sweden
e-mail: mark.dopson@molbiol.umu.se

P. L. Bond
Centre for Ecology, Evolution and Conservation,
University of East Anglia, Norwich NR4 7TJ, UK

Present Address:
C. Baker-Austin
Cefas Weymouth Laboratory,
Weymouth, Dorset DT4 8UB, UK

Present Address:
P. L. Bond
Advanced Water Management Centre,
University of Queensland,
Brisbane, QLD 4072, Australia

Introduction

Biofilms are structured conglomerates of single or multiple species of microorganisms attached to a solid façade or

forming on the surface of a liquid and encased in an extracellular polysaccharide matrix (reviewed in Moons et al. 2009). A wide variety of biofilm-forming acidophiles are present in acidic environments suggesting that a biofilm mode of growth may confer a competitive advantage in these extreme conditions (Moons et al. 2009).

Acidophilic iron-oxidizing microorganisms catalyze the dissolution of sulfide minerals both at the point at which the microorganisms are attached to the mineral (contact, biofilm growth) and via non-contact, planktonic growth (Sand et al. 2001). Metal sulfide oxidation has been exploited in the biohydrometallurgical technique of biomining, where metals are released from sulfide minerals (reviewed in Rohwerder et al. 2003). Surface attachment has been shown to be very important during biomining and is mediated by extracellular polymeric substances (EPS) (reviewed in Vu et al. 2009). The quorum sensing signaling molecules, *N*-acyl homoserine lactones (HSLs) have been identified in acidophilic *Acidithiobacillus* spp. and a quorum sensing locus, including a gene encoding for a AHL synthase, has been identified in the bacterium *Leptospirillum* Group III (*Leptospirillum ferrodiazotrophum*), but not in *Leptospirillum ferrooxidans* (Farah et al. 2005; Rivas et al. 2005, 2007; Ruiz et al. 2008). The transformation to a sessile mode of life is often mediated by intracellular c-di-GMP levels that have been characterized in *Acidithiobacillus* spp. (Castro et al. 2009). A transcriptomic comparison of *A. ferrooxidans* cells attached to pyrite identified several up-regulated outer membrane proteins including a putative pilin (Vera et al. 2009).

'*Ferroplasma acidarmanus*' Fer1 is an extremely acidophilic and metal-tolerant archaeon isolated from the Iron Mountain mine, California, USA (Edwards et al. 2000; Dopson et al. 2004b). '*F. acidarmanus*' Fer1 is one of the most extreme acidophiles isolated to date, and has been shown to grow preferentially encased within mixed population biofilms in the natural environment, constituting up to 85% of a biofilm population community (Bond et al. 2000). Iron Mountain biofilms have low species diversity with a distinct structural organization between *Leptospirillum* spp., archaea including *Ferroplasma* spp., and eukaryotes (Wilmes et al. 2008). The genomes of '*F. acidarmanus*' Fer1 (97% complete; http://genome.ornl.gov/microbial/faci/) and *Ferroplasma* Type II (Tyson et al. 2004) have been sequenced.

In contrast to the acidophile *A. ferrooxidans*, very little is known regarding biofilm formation in the archaeal acidophiles. In this study, we used a combination of bioinformatics, proteomics, and confocal microscopy to investigate biofilm development in '*F. acidarmanus*' Fer1. This study provides further understanding of biofilm formation in an extreme acidophilic archaeon.

Materials and methods

Bioinformatic analysis

The '*F. acidarmanus*' Fer1 genome annotations from the following sites were searched: Integrated Microbial Genomes (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi), Oak Ridge National Laboratory (http://genome.ornl.gov/microbial/faci/), and GenBank (http://www.ncbi.nlm.nih.gov/Genbank/).

The NCBI PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) was searched to determine the known prokaryotic and eukaryotic strategies pertaining to biofilm development that were used to search the '*F. acidarmanus*' Fer1 genome for relevant functions and gene names. A set of known proteins and their homologs from various microbial sources with a documented role in biofilm formation (Supplemental Table S1) was used as bait in a tBLASTn search of '*F. acidarmanus*' Fer1 genome sequence. In addition, predicted proteins annotated as involved in the synthesis of proteinaceous and non-proteinaceous extracellular structures, cell motility, and signal transduction in the related acidophilic archaeon *Picrophilus torridus* DSM 9790 (genome available from the Comprehensive Microbial Resource, http://cmr.jcvi.org/tigr-scripts/CMR/GenomePage.cgi?database=ntpt01; Supplemental Table S2) were used to directly search the GenBank translated '*F. acidarmanus*' Fer1 genome using tBLASTn program. Matching protein candidates were verified using protein BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and comparison with all non-redundant protein sequences deposited in NCBI database.

Strain and growth conditions

The *Ferroplasma* strain was '*F. acidarmanus*' Fer1 (Edwards et al. 2000; Dopson et al. 2004b). All cultures were grown in mineral salts medium (MSM) containing trace elements (Dopson and Lindström 1999), 20 g $FeSO_4{\cdot}7H_2O$ L^{-1}, and 0.02% (wt/vol) yeast extract at 37°C in either batch or air lift continuous culture (Dopson et al. 2005). Growth was followed by measuring protein concentration (Dopson et al. 2004b). The continuous culture was diluted (D) at 0.01 h^{-1} and stirred, with an air flow of 200 mL air min^{-1}. A glass mesh was inserted within the continuous culture vessel to increase the surface area for biofilm development.

Biofilm development in the flow cell

Confocal microscopic analysis of biofilm development was carried out in MSM plus trace elements, Fe^{2+}, and yeast extract (as above). A continuous stream of steady state cells (from the continuous culture) was pumped over the

surface of a polished pyrite chip (pyrite purchased from Ward's) in a flow cell (Supplemental Fig. S1) (Crundwell 1996). Polished pyrite was prepared by embedding pyrite chips (approximately 5 mm in diameter) in epoxy resin. The epoxy cylinder was cut into two parts through the center of the pyrite and the exposed surfaces were polished with fine silicon carbide grinding paper in a LaboPol-6 polisher (Struers). The flow cell was constructed of a polycarbonate base with a distance of 0.5 mm between the base and a glass cover slip held down by an aluminum plate. The circular chamber was 45 mm in diameter and the cell culture was pumped over the surface of the pyrite chip at a flow rate of 6.4 mL min^{-1}. Cells were visualized by addition of 0.00025% to 0.0005% (wt/vol) fluorescein (Sigma-Aldrich) in the continuous culture media, constituting the maximum fluorescein concentration that did not affect '*F. acidarmanus*' Fer1 growth (data not shown). All vessels containing fluorescein were protected from light. Biofilm formation on the pyrite chip was viewed through the glass cover slip. Microscope stage coordinates were used to relocate the same positions on the pyrite chip for comparative analysis of biofilm development over time. The fluorescein-labeled biofilm was imaged with a Zeiss LSM510 Meta confocal microscope using a 40× LD Achroplan objective (N.A. 0.6, correction collar set to 1.5 mm) using a 488 nm argon laser line, and fluorescence was collected using a META detector (emission 526.8–655.2 nm). The pyrite surface was imaged with the same laser in reflectance mode. Images were adjusted and manipulated using Adobe Photoshop. Three-dimensional images were taken for chosen positions on the pyrite chip and the images were analyzed using Velocity software version 3.1 (Improvision).

Protein preparation, two-dimensional polyacrylamide gel electrophoresis, and mass spectrometry

To avoid variations due to differential protein expression during separate growth phases and conditions, protein samples for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) analysis of both planktonic and biofilm modes of growth were prepared simultaneously from cells of a continuous culture vessel sampled during steady state planktonic growth (Dopson et al. 2004a). A glass mesh was inserted into the culture vessel to increase the surface area for biofilm growth. Planktonic cells for proteomic analysis were sampled by draining the medium in the culture vessel from the bottom, while the glass mesh remained inside the reactor. The residual planktonic cells were removed by gentle rinsing with MSM pH 1.2 (as above) before the biofilm cells were removed from the mesh and vessel walls by washing with MSM pH 1.2. Both planktonic and sessile cells were concentrated by separately centrifuging (10,000*g* for 10 min) before storage at −20°C before analysis.

2D-PAGE was performed essentially as previously described (Dopson et al. 2004a). Replicate ($n = 3$) gels were analyzed using ProteomWeaver version 3.0 (Definiens). Protein spot matching, spot presence or absence, and differential expression statistics were determined using ProteomWeaver (Dopson et al. 2005). Protein spots of interest were excised, treated by trypsin, and analyzed using a Bruker Reflex III matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Hesketh et al. 2002; Dopson et al. 2005). MALDI-TOF peptide mass fingerprint data were matched against the '*F. acidarmanus*' Fer1 genome sequence data using MASCOT (http://www.matrixscience.com) and putative protein functions were inferred from analyses contained in the annotated genome site (Dopson et al. 2005).

Detection of *N*-acyl homoserine lactones

Presence of HSLs in cell-free spent media were tested in '*F. acidarmanus*' Fer1 and *Rhizobium* strain J251T as positive control (McClean et al. 1997). Briefly, 1 L '*F. acidarmanus*' Fer1 and 5 mL *Rhizobium* strain J251T were grown until stationary phase under batch conditions in MSM plus trace elements, Fe^{2+}, and yeast extract (as above) and TY media (Rolfe et al. 1980), respectively. Cell-free spent media were extracted with dichloromethane and reconstituted in acetonitrile (McClean et al. 1997). '*F. acidarmanus*' Fer1 and *Rhizobium* J251T organic extracts (5–70 μL) were added to wells in Luria–Bertani (LB) agar plates seeded with *Chromobacterium violaceum* as an indicator organism. LB medium (50 μL) was used as a negative control.

Results and discussion

Genome analysis

The annotated '*F. acidarmanus*' Fer1 genome was examined for proteins with high sequence similarity to those known to be involved in biofilm formation and maintenance in several different microbial species (Supplemental Table S1). A number of candidate genes were identified (Supplemental Table S2). These included putative genes for motility and attachment including flagella and pilus biogenesis; and Type II, III, and IV secretory pathway proteins for transport of organic molecules to the cell surface (reviewed in Donlan (2002)). The genome encoded numerous putative proteins predicted to be involved in the synthesis of surface polysaccharides, including two cellulose synthases, which may affect the architecture of the

'*F. acidarmanus*' Fer1 biofilm matrix (reviewed in Karatan and Watnick (2009)). Membrane proteases were also identified which may be important for biofilm signaling, maturation, or dispersal (reviewed in Karatan and Watnick (2009)). In addition, the '*F. acidarmanus*' Fer1 genome encodes proteins predicted to be involved in polyamine synthesis, which in some microorganisms have been shown to regulate surface-associated growth and biofilm formation (Karatan et al. 2005).

However, no AHL-binding transcriptional regulators (LuxR homologs) or putative HSL synthases were identified that are commonly associated with biofilm formation in Gram-negative acidophiles. Although all components of the basic activated methyl cycle were present (i.e. methionine synthase, *S*-adenosyl-L-methionine synthetase, SAM-dependent methyltransferases, and *S*-adenosyl-L-homocysteine hydrolase), Pfs and LuxS required for the synthesis of the universal quorum signal AI-2 were not evident (Henke and Bassler 2004). Furthermore, no members of the bacterial c-di-GMP signaling system (proteins bearing the GGDEF or EAL domains; Simm et al. 2004) were identified and no genetic evidence for bacterial and fungal non-ribosomal quorum peptides was found (Bushley et al. 2008; Bushley and Turgeon 2010). '*F. acidarmanus*' Fer1 is unlikely to respond to HSLs from other species as the genome does not contain a LuxR homolog or other quorum sensing signal molecule receptors. Nevertheless, these results do not conclusively preclude the possibility that '*F. acidarmanus*' Fer1 is able to detect and respond to other types of as yet unidentified signaling molecules synthesized by other microorganisms.

Biofilm morphologies

Two different '*F. acidarmanus*' Fer1 biofilm morphologies were observed: a multi-layer film that formed on both the inert glass as well as the pyrite that acts as energy source (Fig. 1), and ~5 mm-long filaments that formed on the sintered glass spargers in the gas lift bioreactors (unrecorded observation). These morphologies were reminiscent of the pellicle (Wilmes et al. 2008) and filamentous (Edwards et al. 2000) natural biofilms originally observed at the Iron Mountain mine. The formation of two different types of single species biofilm suggests that '*F. acidarmanus*' Fer1 does not require extraneous signaling molecules from other species to modify the architecture of the biofilm it forms, a conclusion supported by the lack of known quorum sensing signal molecule receptors.

Confocal microscopic analysis of biofilm development

Biofilm development was investigated on the mineral surface in a flow cell under conditions relevant to those in metal leaching environments. After 3 days, only planktonic cells from the steady state continuous culture reactor were observed (Fig. 1a), after 10 and 21 days microcolonies were identified (Fig. 1b, c), and after 38 days the entire surface of the pyrite chip was covered in biofilm (Fig. 1d). The rate of attachment to mineral under laboratory flow cell conditions was slower for '*F. acidarmanus*' Fer1 than a mixed culture of *A. ferrooxidans* and *L. ferrooxidans* oxidizing microorganisms that formed a biofilm on pyrite after 2–5 days (Crundwell 1996) and an *A. ferrooxidans* biofilm that covered the surface of chalcopyrite after 14–21 days (Fu et al. 2004). The slower biofilm formation rate of '*F. acidarmanus*' Fer1 might be due to its longer generation time which is approximately an order of magnitude greater than *A. ferrooxidans* (Golyshina and Timmis 2005).

Proteomic analysis of '*F. acidarmanus*' Fer1 biofilm growth

To avoid variations due to differential protein expression during separate growth phases and conditions, protein samples for 2D-PAGE analysis of both planktonic and biofilm modes of growth were prepared simultaneously from cells of a continuous culture vessel sampled during steady state planktonic growth (Dopson et al. 2004a). 2D-PAGE protein expression profiles between a 44-day-old biofilm formed on glass and planktonic cells were compared (Fig. 2). However, it should be noted that glass,

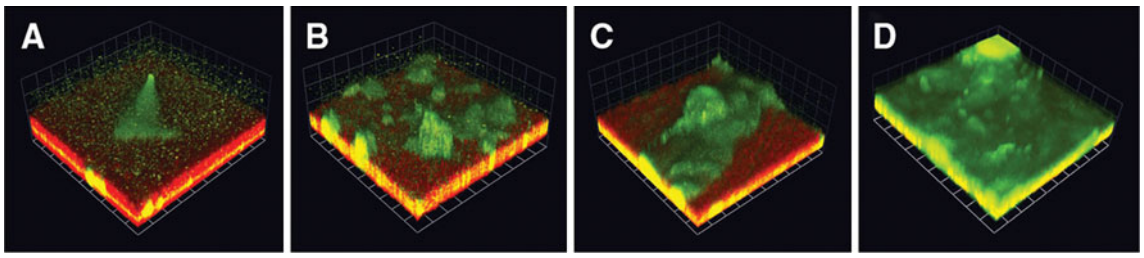

Fig. 1 Representative confocal images of '*F. acidarmanus*' Fer1 biofilm development (image sizes are 230 × 230 μm). Images were produced after 3 (**a**), 10 (**b**), 21 (**c**), and 38 days (**d**) of development on the pyrite surface. Four separate positions on the pyrite chip were assayed for each time point. '*F. acidarmanus*' Fer1 cells were stained in *green* while the pyrite surface is depicted in *red*

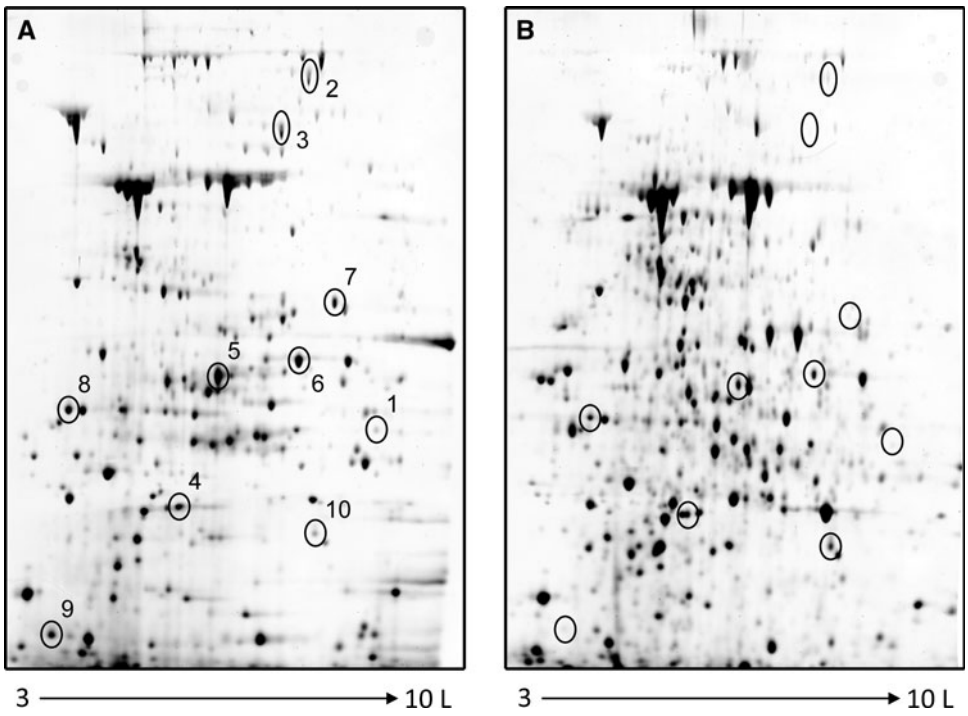

Fig. 2 2D-PAGE gels of the proteomic response to biofilm growth. Images are representative gels from biofilm (**a**) and planktonic (**b**) grown '*F. acidarmanus*' Fer1. Apparent protein spot intensities may not exactly match those in Table 1 (stimulation) as mean values were calculated from replicate gels ($n = 3$). Spot numbers refer to Table 1

unlike pyrite, does not constitute an energy source for the '*F. acidarmanus*' Fer1 cells. Therefore, the biofilm structure and protein expression may differ to that generated if a sulfide mineral surface had been used.

Protein spots up-regulated ≥4 fold under biofilm conditions were excised and analyzed for putative identification according to the draft genome analysis (Table 1 and further details in Supplemental Table S3). Four proteins up-regulated during biofilm growth were also previously detected as up-regulated during '*F. acidarmanus*' Fer1 anaerobic growth (Dopson et al. 2007). These were a subunit of pyruvate ferredoxin oxidoreductase that catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA and CO_2 in anaerobic microorganisms (Ma et al. 1997); an electron transfer flavoprotein that is utilized during anaerobic carnitine metabolism in *Escherichia coli* (Walt and Kahn 2002); a RNase exoribonuclease; and a hypothetical protein. In addition, two further proteins associated with anaerobic growth were identified (Table 1). These were malate dehydrogenase that participates in reversed electron transport for anaerobic energy production and thioredoxin reductase that catalyzes the transfer of electrons from NADH to thioredoxin in some anaerobic bacteria (Harms et al. 1998). The up-regulated alkyl hydroperoxide reductase is the main scavenger for H_2O_2 in *E. coli* (Seaver and Imlay 2001) and may be up-regulated in response to the presence of free iron. Also, *S*-adenosylmethionine synthetase that produces *S*-adenosylmethionine (SAM) was up-regulated. Interestingly, in addition to several other functions, SAM is an amino propyl donor for polyamine biosynthesis (Yocum et al. 1996) involved in biofilm formation.

In conclusion, the majority of the up-regulated proteins identified from the biofilm were associated with anaerobic growth. It has been previously observed that underlying sections of biofilms can become anaerobic (Davey and O'Toole 2000) and a proteomic analysis of a *Bacillus cereus* mature biofilm also identified proteins associated with anaerobic growth (Oosthuizen et al. 2002). No proteins predicted to be involved in the initial stages of biofilm formation were identified, potentially due to that a 44-day-old biofilm was analyzed to obtain sufficient biomass for the analysis or the use of a glass surface for biofilm growth.

Analysis for *N*-acyl homoserine lactones

Many microorganisms utilize HSLs to mediate biofilm formation, as has been suggested for the acidophile *A. ferrooxidans* (Farah et al. 2005; Rivas et al. 2005, 2007; Ruiz et al. 2008). Consequently, we assayed for the presence of mainly short-chain HSLs in culture supernatant using the indicator organism *Chromobacterium violaceum*. Evidence for HSL production has been reported for a single archaeon (Paggi et al. 2003), but no evidence for HSL production by '*F. acidarmanus*' Fer1 was detected (Supplemental Fig. S2). This result was in agreement with our bioinformatic analysis of the '*F. acidarmanus*' Fer1 genome, which suggested that there were no genes with high sequence similarity to those encoding Gram-negative bacterial quorum sensing system components (Supplemental Tables S1 & S2).

Table 1 Identification of '*F. acidarmanus*' Fer1 proteins induced in a 44-day-old biofilm

Gene[a]	Putative protein function	Spot[b]	Stimulation[c]	Average ± SD[d]
Metabolic and electron transport proteins				
ZP_05571540	Malate dehydrogenase Mdh	1	NP[e]	0.55 ± 0.07
ZP_05571387	Pyruvate ferredoxin oxidoreductase (α-subunit)	2	19.3	1.08 ± 0.78
ZP_05570017	Electron transfer flavoprotein (α-subunit)	3	8.1	0.39 ± 0.10
ZP_05571476	Aerobic-type carbon monoxide dehydrogenase	4	6.9	1.01 ± 0.33
ZP_05571531	Succinyl-CoA synthetase (SucC), *β*-subunit	5	6.4	1.52 ± 0.52
ZP_05570511	Thioredoxin reductase, TrxB	6	4.6	2.01 ± 0.61
Biosynthetic proteins				
ZP_05570185	*S*-adenosylmethionine synthetase	7	NP	0.64 ± 0.08
Transcription and translation components				
ZP_05570806	RNase exoribonuclease	8	7.6	0.75 ± 0.09
Stress proteins and chaperones				
ZP_05570082	Peroxiredoxin-alkyl hydroperoxide reductase subunit C	9	NP	0.43 ± 0.07
Other proteins				
ZP_05570006	Hypothetical protein	10	12.1	0.34 ± 0.13

[a] Designates the NCBI accession number for the protein as identified on the '*F. acidarmanus*' Fer1 draft genome analysis (http://www.ncbi.nlm.nih.gov/)

[b] Spot numbers correspond to the labeled spots on Fig. 2

[c] Stimulation refers to the increase in protein expression determined by comparison of gel sets using ProteomWeaver

[d] Average ± SD of normalized spot intensity of the technical replicates calculated by ProteomWeaver

[e] *NP*, designates the spot is not present in the corresponding gel set

However, since the longer chain HSLs are not detected by the indicator *C. violaceum*, their production may not be conclusively ruled out.

Conclusions

Two different '*F. acidarmanus*' Fer1 biofilm morphologies were observed that mimicked those encountered in the natural environment. Bioinformatic analysis identified genes for flagella and EPS formation important in motility and attachment. Biofilm development in '*F. acidarmanus*' Fer1 was analyzed on pyrite surfaces in a flow cell fed with steady state growth cells from a continuous culture reactor. Proteomic analysis revealed that 6 out of the 10 identified up-regulated proteins were involved in anaerobic growth suggesting that internal zones of the biofilm were anaerobic. No evidence was found for quorum sensing and the '*F. acidarmanus*' Fer1 signaling molecule for biofilm formation remains elusive.

Acknowledgments Drs. Jillian F. Banfield and Katrina J. Edwards are gratefully acknowledged for provision of '*F. acidarmanus*' Fer1 and helpful discussions. Polished pyrite chips were kindly prepared by Åke Sandström. The technical assistance of Martina Maidment and Lynda Flegg was greatly appreciated and Dr. Paul Thomas is acknowledged for assistance with the confocal microscopy. Trypsin digests and MALDI-TOF analysis of excised protein spots was carried out at the John Innes Centre, Norwich. This work was funded by a BBSRC Research grant and the John and Pamela Salter Charitable Trust.

References

Bond PL, Smriga SP, Banfield JF (2000) Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. Appl Environ Microbiol 66:3842–3849

Bushley KE, Turgeon BG (2010) Phylogenomics reveals subfamilies of fungal nonribosomal peptide synthetases and their evolutionary relationships. BMC Evol Biol 10:26

Bushley KE, Ripoll DR, Turgeon BG (2008) Module evolution and substrate specificity of fungal nonribosomal peptide synthetases involved in siderophore biosynthesis. BMC Evol Biol 8:328

Castro M, Ruiz LM, Barriga A, Jerez CA, Holmes D, Guiliani N (2009) C-di-GMP pathway in biomining bacteria. Adv Mat Res 71–73:223–226

Crundwell F (1996) The formation of biofilms of iron-oxidising bacteria on pyrite. Miner Eng 9:1081–1089

Davey ME, O'Toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890

Dopson M, Lindström EB (1999) Potential role of *Thiobacillus caldus* in arsenopyrite bioleaching. Appl Environ Microbiol 65:36–40

Dopson M, Baker-Austin C, Bond PL (2004a) First use of 2-dimensional polyacrylamide gel electrophoresis to determine phylogenetic relationships. J Microbiol Methods 58:297–302

Dopson M, Baker-Austin C, Hind A, Bowman JP, Bond PL (2004b) Characterization of *Ferroplasma* isolates and *Ferroplasma acidarmanus* sp nov., extreme acidophiles from acid mine drainage and industrial bioleaching environments. Appl Environ Microbiol 70:2079–2088

Dopson M, Baker-Austin C, Bond PL (2005) Analysis of differential protein expression during growth states of *Ferroplasma* strains and insights into electron transport for iron oxidation. Microbiology 151:4127–4137

Dopson M, Baker-Austin C, Bond P (2007) Towards determining details of anaerobic growth coupled to ferric iron reduction by the acidophilic archaeon '*Ferroplasma acidarmanus*' Fer1. Extremophiles 11:159–168

Edwards KJ, Bond PL, Gihring TM, Banfield JF (2000) An archaeal iron-oxidizing extreme acidophile important in acid mine drainage. Science 287:1796–1799

Farah C, Vera M, Morin D, Haras D, Jerez CA, Guiliani N (2005) Evidence for a functional quorum-sensing type AI-1 system in the extremophilic bacterium *Acidithiobacillus ferrooxidans*. Appl Environ Microbiol 71:7033–7040

Fu JH, Hu YH, Qiu GZ, Liu JS, Xu J (2004) Biofilm forming and leaching mechanism during bioleaching chalcopyrite by *Thiobacillus ferrooxidans*. Trans Nonferr Metals Soc China 14:383–387

Golyshina OV, Timmis KN (2005) *Ferroplasma* and relatives, recently discovered cell wall-lacking archaea making a living in extremely acid, heavy metal-rich environments. Environ Microbiol 7:1277–1288

Harms C, Meyer MA, Andreesen JR (1998) Fast purification of thioredoxin reductases and of thioredoxins with an unusual redox-active centre from anaerobic, amino-acid-utilizing bacteria. Microbiology 144:793–800

Henke JM, Bassler BL (2004) Bacterial social engagements. Trends Cell Biol 14:648–656

Hesketh A et al (2002) The GlnD and GlnK homologues of *Streptomyces coelicolor* A3(2) are functionally dissimilar to their nitrogen regulatory system counterparts from enteric bacteria. Mol Microbiol 46:319–330

Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73:310–347

Karatan E, Duncan TR, Watnick PI (2005) *NspS*, a predicted polyamine sensor, mediates activation of *Vibrio cholerae* biofilm formation by norspermidine. J Bacteriol 187:7434–7443

Ma K, Hutchins A, Sung S-JS, Adams MWW (1997) Pyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon, *Pyrococcus furiosus*, functions as a CoA-dependent pyruvate decarboxylase. Proc Natl Acad Sci USA 94:9608–9613

McClean K et al (1997) Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of *N*-acylhomoserine lactones. Microbiology 143:3703–3711

Moons P, Michiels CW, Aertsen A (2009) Bacterial interactions in biofilms. Crit Rev Microbiol 35:157–168

Oosthuizen MC et al (2002) Proteomic analysis reveals differential protein expression by *Bacillus cereus* during biofilm formation. Appl Environ Microbiol 68:2770–2780

Paggi RA, Martone CB, Fuqua C, De Castro RE (2003) Detection of quorum sensing signals in the haloalkaliphilic archaeon *Natronococcus occultus*. FEMS Microbiol Lett 221:49–52

Rivas M, Seeger M, Holmes DS, Jedlicki E (2005) A lux-like quorum sensing system in the extreme acidophile *Acidithiobacillus ferrooxidans*. Biol Res 38:283–297

Rivas M, Seeger M, Jedlicki E, Holmes DS (2007) Second acyl homoserine lactone production system in the extreme acidophile *Acidithiobacillus ferrooxidans*. Appl Environ Microbiol 73:3225–3231

Rohwerder T, Gehrke T, Kinzler K, Sand W (2003) Bioleaching review part A. Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Appl Microbiol Biotechnol 63:239–248

Rolfe BG, Gresshoff PM, Shine J (1980) Rapid screening for symbiotic mutants of *Rhizobium* and white clover. Plant Sci Lett 19:277–284

Ruiz LM et al (2008) AHL communication is a widespread phenomenon in biomining bacteria and seems to be involved in mineral-adhesion efficiency. Hydrometallurgy 94:133–137

Sand W, Gehrke T, Jozsa P-G, Schippers A (2001) (Bio)chemistry of bacterial leaching-direct vs indirect bioleaching. Hydrometallurgy 59:159–175

Seaver LC, Imlay JA (2001) Alkyl hydroperoxide reductase is the primary scavenger of endogenous hydrogen peroxide in *Escherichia coli*. J Bacteriol 183:7173–7181

Simm R, Morr M, Kader A, Nimtz M, Romling U (2004) GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. Mol Microbiol 53:1123–1134

Tyson GW et al (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428:37–43

Vera M, Rohwerder T, Bellenberg S, Sand W, Denis Y, Bonnefoy V (2009) Characterization of biofilm formation by the bioleaching acidophilic bacterium *Acidithiobacillus ferrooxidans* by a microarray transcriptome analysis. Adv Mat Res 71–73:175–178

Vu B, Chen M, Crawford RJ, Ivanova EP (2009) Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 14:2535–2554

Walt A, Kahn ML (2002) The *fixA* and *fixB* genes are necessary for anaerobic carnitine reduction in *Escherichia coli*. J Bacteriol 184:4044–4047

Wilmes P, Remis JP, Hwang M, Auer M, Thelen MP, Banfield JF (2008) Natural acidophilic biofilm communities reflect distinct organismal and functional organization. ISME J 3:266–270

Yocum RR, Perkins JB, Howitt CL, Pero J (1996) Cloning and characterization of the *metE* gene encoding S-adenosylmethionine synthetase from *Bacillus subtilis*. J Bacteriol 178:4604–4610