

Supplementary Information

Methods

Growth Characterisation

Total Adenosine Triphosphate (ATP) was determined using a Molecular Probes ATP Determination Kit (Life Technologies, USA). 0.5 ml of sample was diluted with 0.5 ml 1 mol.L⁻¹ Tris-EDTA buffer (pH 7.2), and the cells were then lysed by heating to 90 °C in a Labnet Accublock digital dry bath for 15 min. The lysate was then centrifuged at 13,300 × *g* for 15mins. ATP in the supernatant was measured using a luciferin luciferase assay, with the luminescence determined on a Glomax 20/20 luminometer (Promega, USA).

DNA Extraction and sequencing of the 16S rRNA Gene

DNA was extracted from the bacterial community growing in the alkaline Fe(III) media containing yeast extract as the only source of electron donors using a FastDNA spin kit for soils (MP Biomedicals, USA). Cultures (1.5 ml) were centrifuged at 7000 × *g* for 15 min, and the supernatant discarded. The cell pellet was resuspended in sodium phosphate buffer, and transferred to the tubes containing the lysing matrix. The standard FastDNA protocol was then followed.

A 1.5 kb fragment of the 16s rRNA gene was amplified by Polymerase Chain Reaction (PCR) using broad specificity primers (8f: AGAGTTTGATCCTGGCTCAG (1), and 1492r: ACGGYTACCTTGTTACGACTT, where Y = C or T (2)). Each PCR reaction mixture contained 5 µl of purified DNA, 5 units GoTaq DNA polymerase (Promega Corp., USA), 1× PCR reaction buffer, 2.0 mM MgCl₂, 0.2 mM PCR nucleotide mix (Promega Corp., USA), and 0.6 µM DNA primers in a final volume of

50 μ l. The reaction mixtures were incubated at 95 °C for 2 min, and then cycled 30 times through three steps: denaturing (95°C, 45 s), annealing (48°C, 60 s), primer extension (72°C, 120 s). This was followed by a final extension step at 72°C for 4 min.

The 1.5 kb PCR product was isolated using an agarose-TBE gel and extracted using a QIAquick gel extraction kit. The PCR product was ligated into pGEM-T Easy (Promega Corp., USA), and transformed into *E.coli* XL-1 blue competent cells (Agilent Technologies UK Ltd). The cells were grown on LB-agar plates containing ampicillin (100 μ g/ml) before colonies were selected and sent for sequencing (GATC Biotech Ltd, Germany).

Cells from colonies isolated on agar plates were transferred into 100 μ l ddH₂O using a sterile toothpick. DNA was extracted from the cell suspension using a FastDNA spin kit for soils. A 1.5 kb fragment of the 16S rRNA gene was amplified by PCR, and isolated on an agarose-TBE gel (see above). The PCR product was sent for direct sequencing.

The quality of gene sequences was evaluated using Mallard 1.02 (3), and putative chimeras were excluded from subsequent analyses. Sequences were classified using the Ribosomal Database Project (RDP) naïve Bayesian Classifier (4). Sequences were grouped into operational taxonomic units (OTUs) using the MOTHUR software (>98% nearest neighbour sequence similarity cut-off) (5). Phylogenetic trees were constructed using representative sequences from selected OTUs, aligned with type species from the EMBL database using ClustalX (v2.0) (6), and drawn with TreeView (v1.6.6) (7). Trees were constructed from the distance matrix by neighbour joining, with bootstrap analysis performed with 2000 replicates in order to achieve >95% repeatability.

Results

Bacterial Growth Curves

The growth phase of the iron reducing culture can be modelled using a logistic sigmoidal growth function (8);

$$y = \frac{A}{\left\{1 + \exp\left[\frac{\mu_m}{A}(\lambda - t) + 2\right]\right\}} \quad (1)$$

Where A= average maximum cell numbers (cells/ml), μ_m = maximum specific growth rate (unit), λ = lag time (hours) and t = time (hours). This growth function was to fit the cell numbers in **Figure 1a** using $A = 166.7 \times 10^6$ cells/l, $\lambda = 72$ hours and $\mu_m = 0.6 \times 10^6$ cell/l/h. The same growth function was used to fit the total Fe(II) data for the population in AFC media in **Figure 1c** and **Figure 6** using the average maximum amount of Fe(II) (3500 μ M) for A, and a maximum specific reduction rate of 29 μ M/h for μ_m , and a lag time of 96 hours. The same function was fitted to the total Fe(II) data for the population in AFC + riboflavin media in **Figure 6** with $A = 3500\mu$ M, $\lambda = 48$ hours and $\mu_m = 41 \mu$ M/h.

References

1. **Eden PA, Schmidt TM, Blakemore RP, Pace NR.** 1991. *Phylogenetic Analysis of Aquaspirillum magnetotacticum Using Polymerase Chain Reaction-Amplified 16S rRNA-Specific DNA*. Int. J. Syst. Bacteriol., **41**(2):324-325.
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6. **Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG.** 2007. *Clustal W and clustal X version 2.0.* Bioinformatics, **23**(21):2947-2948.
7. **Page RDM.** 1996. *TreeView: An application to display phylogenetic trees on personal computers.* Comput. Appl. Biosci., **12**(4):357-358.
8. **Zwietering MH, Jongenburger I, Rombouts FM, van 't Riet K.** 1990. *Modeling of the Bacterial Growth Curve.* Appl. Envir. Microbiol., **56**(6):1875-1881.

Table S1. RDP classification with 95% confidence threshold and OTU assignment for sequences obtained from iron reducing consortium.

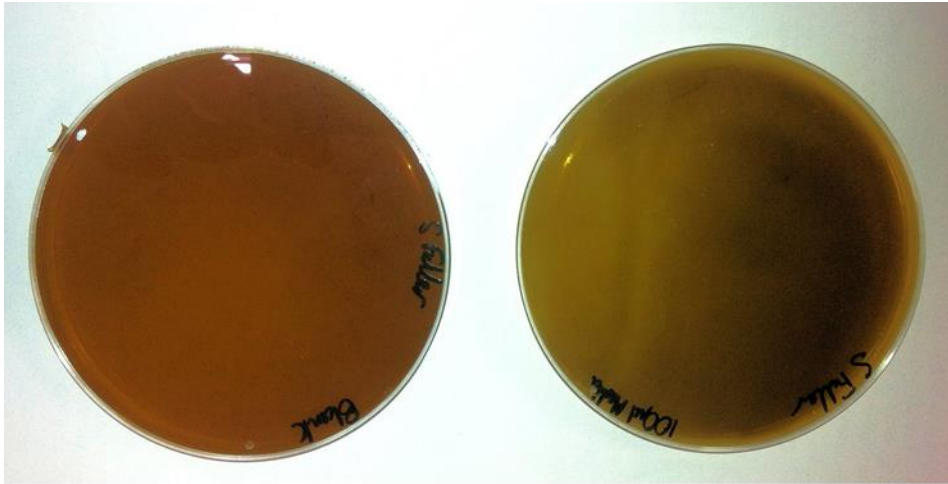
ID	Accession Number	Sequence length	Classification using the RDP classifier (95% Confidence threshold)	OTU
Fe1	KF362050	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe2	KF362051	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe3	KF362052	1466	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe4	KF362053	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe5	KF362054	1495	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella C
Fe6	KF362055	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe8	KF362056	1489	Firmicutes, Clostridia, Clostridiales, Clostridiaceae 2, Alkaliphilus	Alkaliphilus
Fe9	KF362057	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe10	KF362058	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe11	KF362059	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe12	KF362060	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe14	KF362061	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe15	KF362062	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe16	KF362063	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe17	KF362064	1496	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella A
Fe18	KF362065	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe19	KF362066	1496	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella B
Fe20	KF362067	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe21	KF362068	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe22	KF362069	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe25	KF362070	1496	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella B
Fe26	KF362071	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe27	KF362072	1496	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella A
Fe28	KF362073	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe29	KF362074	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe30	KF362075	1488	Firmicutes, Clostridia, Clostridiales, Clostridiaceae 2, Alkaliphilus	Alkaliphilus
Fe31	KF362076	1496	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella B
Fe32	KF362077	1468	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe33	KF362078	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe34	KF362079	1495	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella C
Fe35	KF362080	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe36	KF362081	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe37	KF362082	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe39	KF362083	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe41	KF362084	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe42	KF362085	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe43	KF362086	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe44	KF362087	1487	Firmicutes, Clostridia, Clostridiales, Clostridiaceae 2, Alkaliphilus	Alkaliphilus
Fe45	KF362088	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe47	KF362089	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe48	KF362090	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe49	KF362091	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe50	KF362092	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe51	KF362093	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe52	KF362094	1489	Firmicutes, Clostridia, Clostridiales, Clostridiaceae 2, Alkaliphilus	Alkaliphilus
Fe53	KF362095	1494	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe54	KF362096	1466	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe55	KF362097	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe56	KF362098	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe57	KF362099	1466	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe58	KF362100	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe59	KF362101	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe60	KF362102	1465	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe61	KF362103	1496	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella A
Fe63	KF362104	1495	Firmicutes, Clostridia, Clostridiales	Tissierella C
Fe64	KF362105	1496	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella	Tissierella A
Fe65	KF362106	1489	Firmicutes, Clostridia, Clostridiales, Clostridiaceae 2, Alkaliphilus	Alkaliphilus
Fe66	KF362107	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI
Fe68	KF362108	1467	Firmicutes, Clostridia, Clostridiales, Peptostreptococcaceae, Clostridium XI	Clostridium XI

Table S2. RDP classification with 95% confidence threshold for sequences obtained from streaks on agar plates which cleared the surrounding gel.

ID	Accession Number	Sequence length	Classification using the RDP classifier (95% Confidence threshold)
Str5	KF362109	1402	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella
Str6	KF362110	1315	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella
Str9	KF362111	1234	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella
Str20	KF362112	1398	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella
Str26	KF362113	1400	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XI, Tissierella

Table S3. RDP classification with 95% confidence threshold for sequences obtained from streaks on agar plates which did not clear the surrounding gel.

ID	Accession Number	Sequence length	Classification using the RDP classifier (95% Confidence threshold)
Str3	KF362114	1304	Actinobacteria, Actinobacteridae, Actinomycetales
Str4	KF362115	462	Actinobacteria, Actinobacteridae, Actinomycetales
Str10	KF362116	515	Actinobacteria, Actinobacteridae, Actinomycetales
Str48	KF362117	1359	Proteobacteria, Alphaproteobacteria, Rhizobiales, Brucellaceae, Ochrobactrum



Media

Media post growth

Figure S1. Anaerobic growth of the iron reducing consortia on AFC media-agar plates.