Assessing the Diversity of Anaerobes in Acidic Environments Using a Combination of Culture-based and Cultivation-independent Methods

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Biological Diversity in Ecosystems

Ecosystem with versatile living conditions:

→ high diversity

→ low numbers of individuals

Ecosystem with one-sided living conditions:

→ low diversity

→ high number of individuals

Thienemann 1920

Microbial Diversity and Abundance of Species

Discrepancy between:

Number of bacterial colonies that from on solid media and the total number of bacteria

Cutler and Crump 1935

Application of molecular ecological techniques:

comparative analysis of 16S rRNAs or 16S rRNA genes

qualitative and some quantitative informations

“Unculturable” Bacteria

Inadequate traditional cultivation techniques ↔ More sophisticated molecular techniques

Bioremediation potential of bacteria?

⇓

Isolate abundant species

Isolation of phylogenetically novel soil bacteria

Joseph et al 2003

Acidic Coal Mining Lake Sediments

Formation of Acidity in Coal Mining Lakes

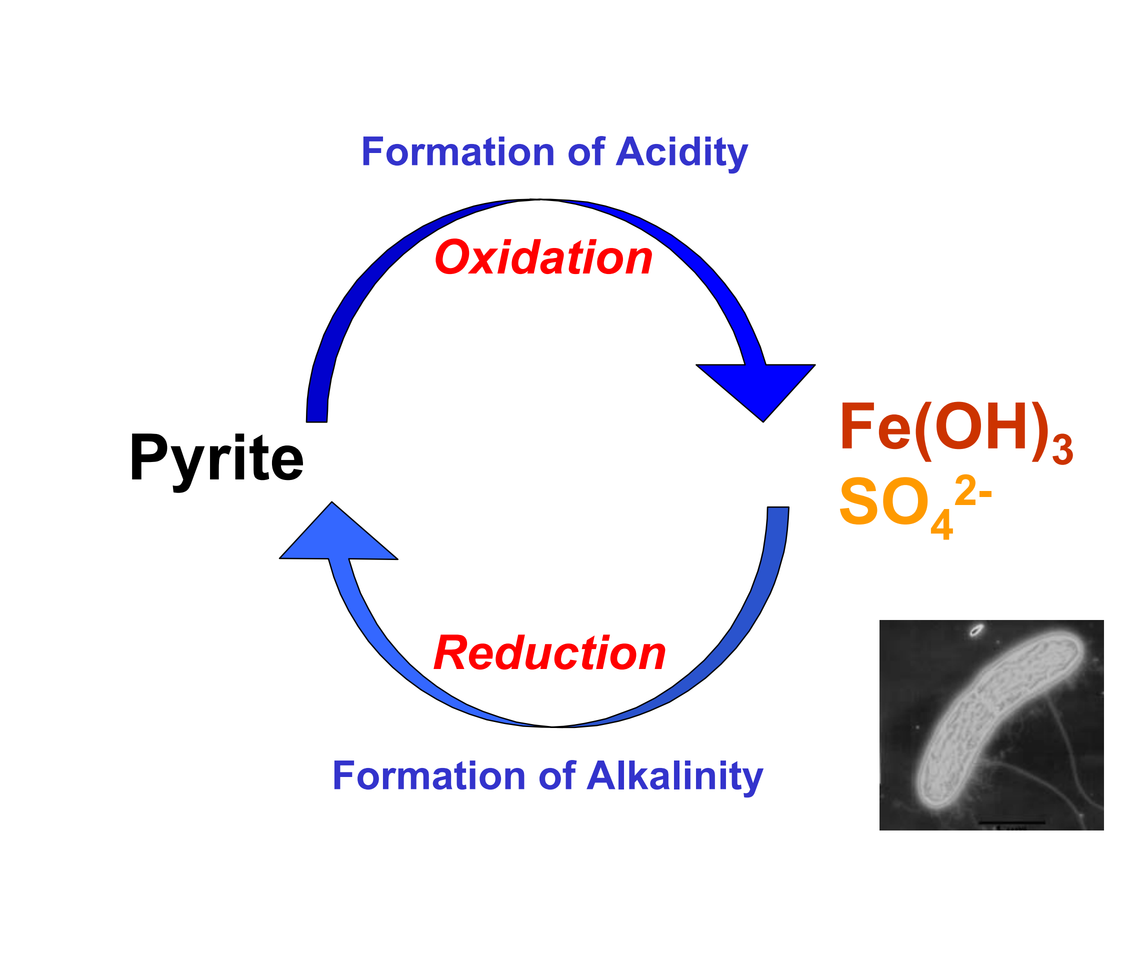
Oxidation of Pyrite

$FeS_2 + 3.5\ O_2 + H_2O \rightarrow Fe^{2+} + 2\ SO_4^{2-} + 2\ H^+$

Characteristics of Acidic Coal Mining Lakes

Oxidation of Pyrite $FeS_2 + 3.5\ O_2 + H_2O \rightarrow Fe^{2+} + 2\ SO_4^{2-} + 2\ H^+$

Can we reverse these processes?



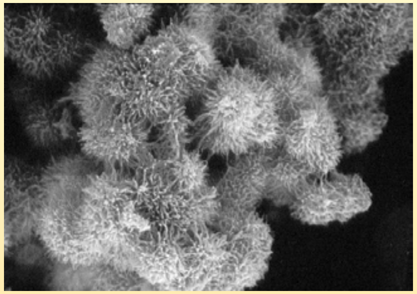
Fe(III) reducers

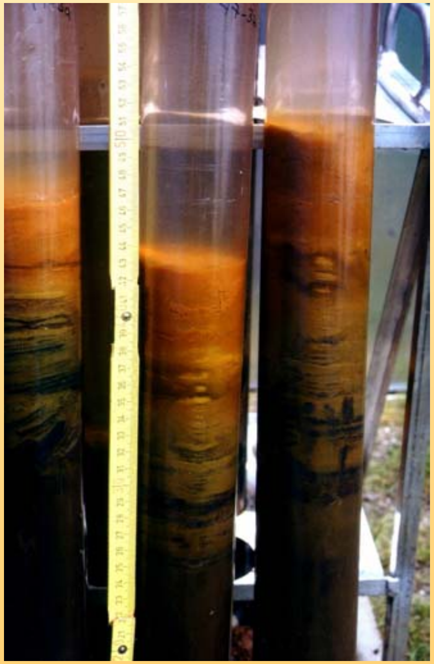
Sulfate reducers

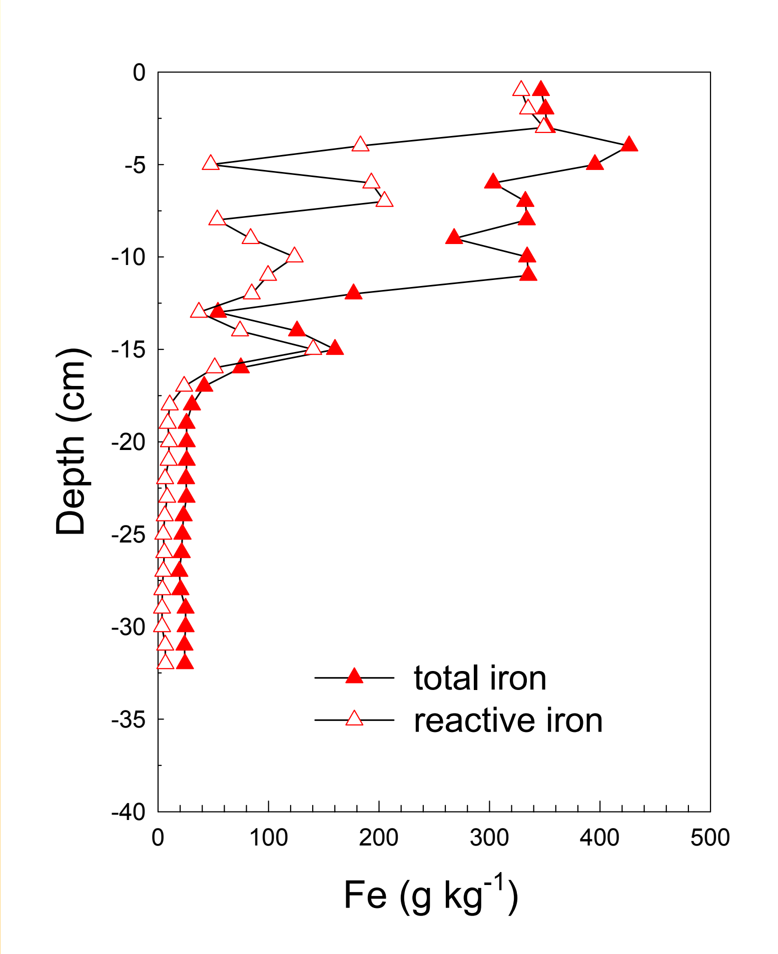
Depth Profiles of the Sediment Solid Phase

Schwertmannite

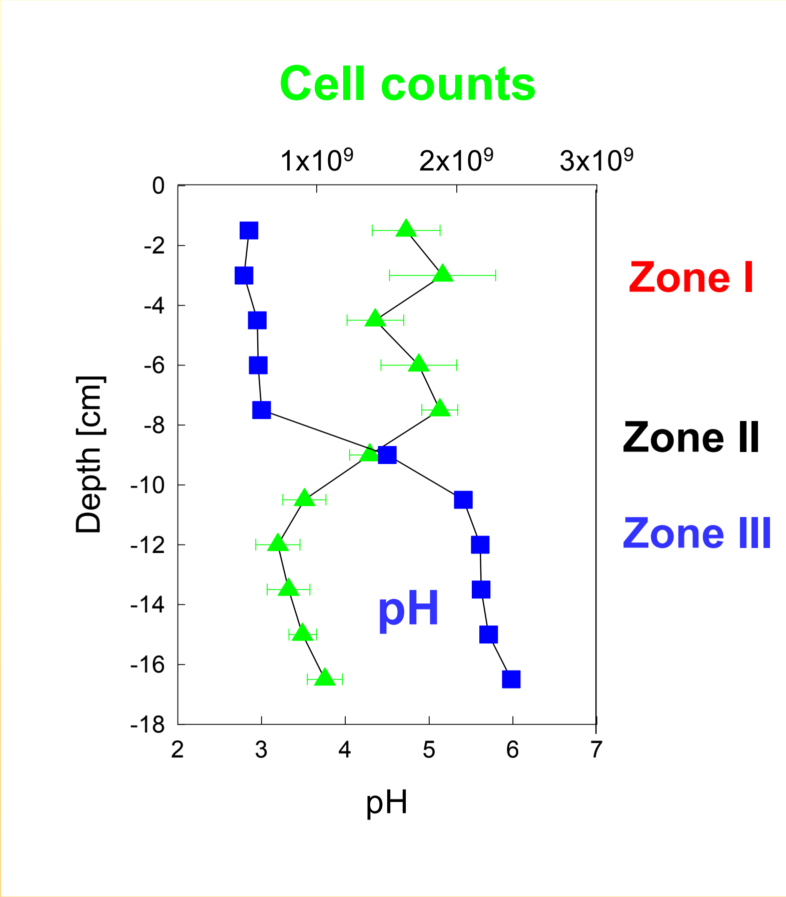
$Fe_8O_8(OH)_x(SO_4)_y$

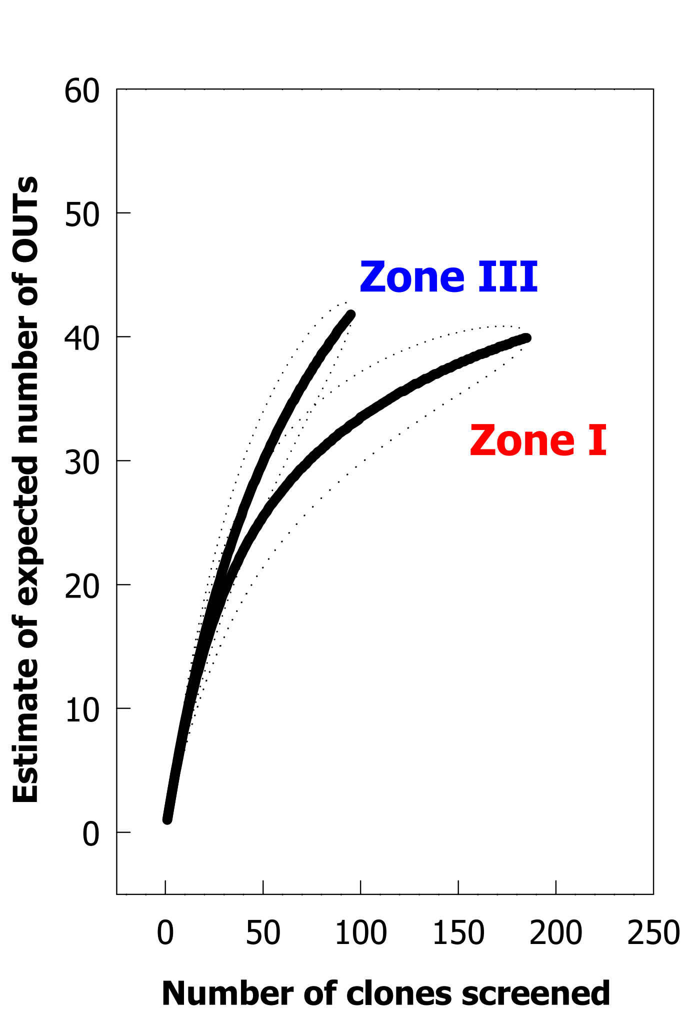




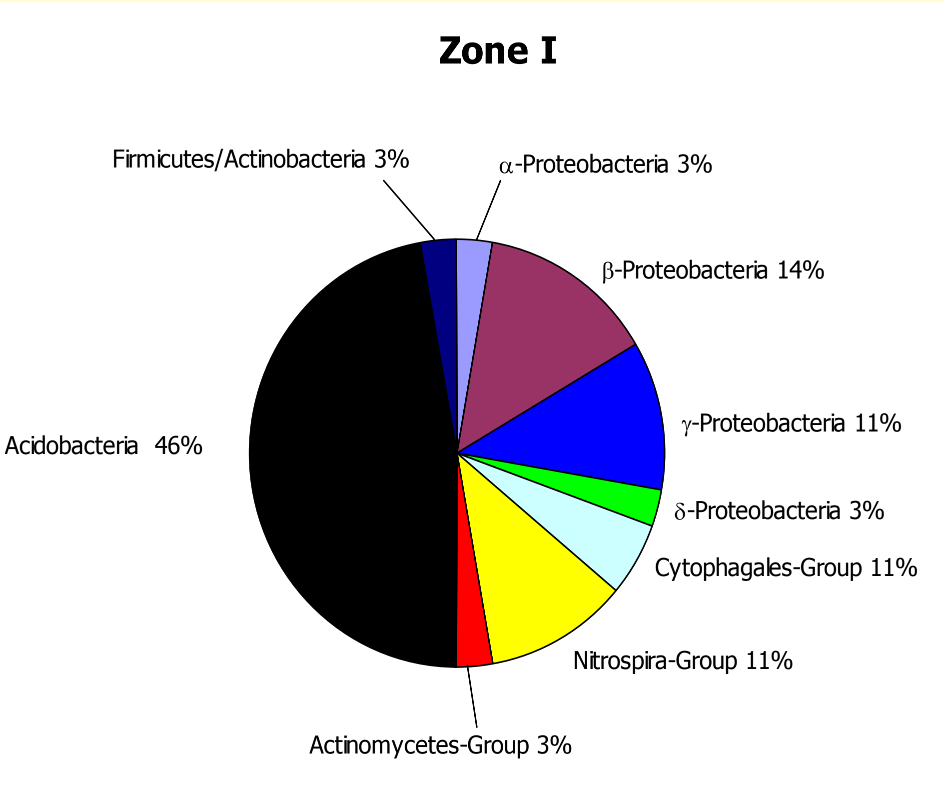


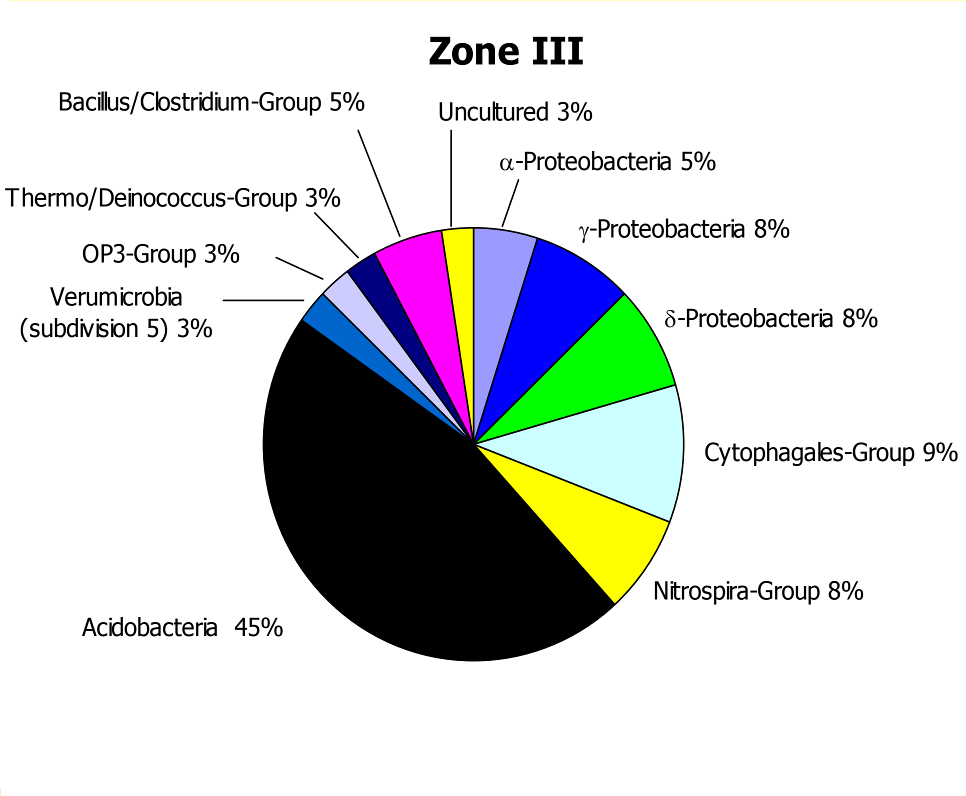
Do we have different microbial communities?





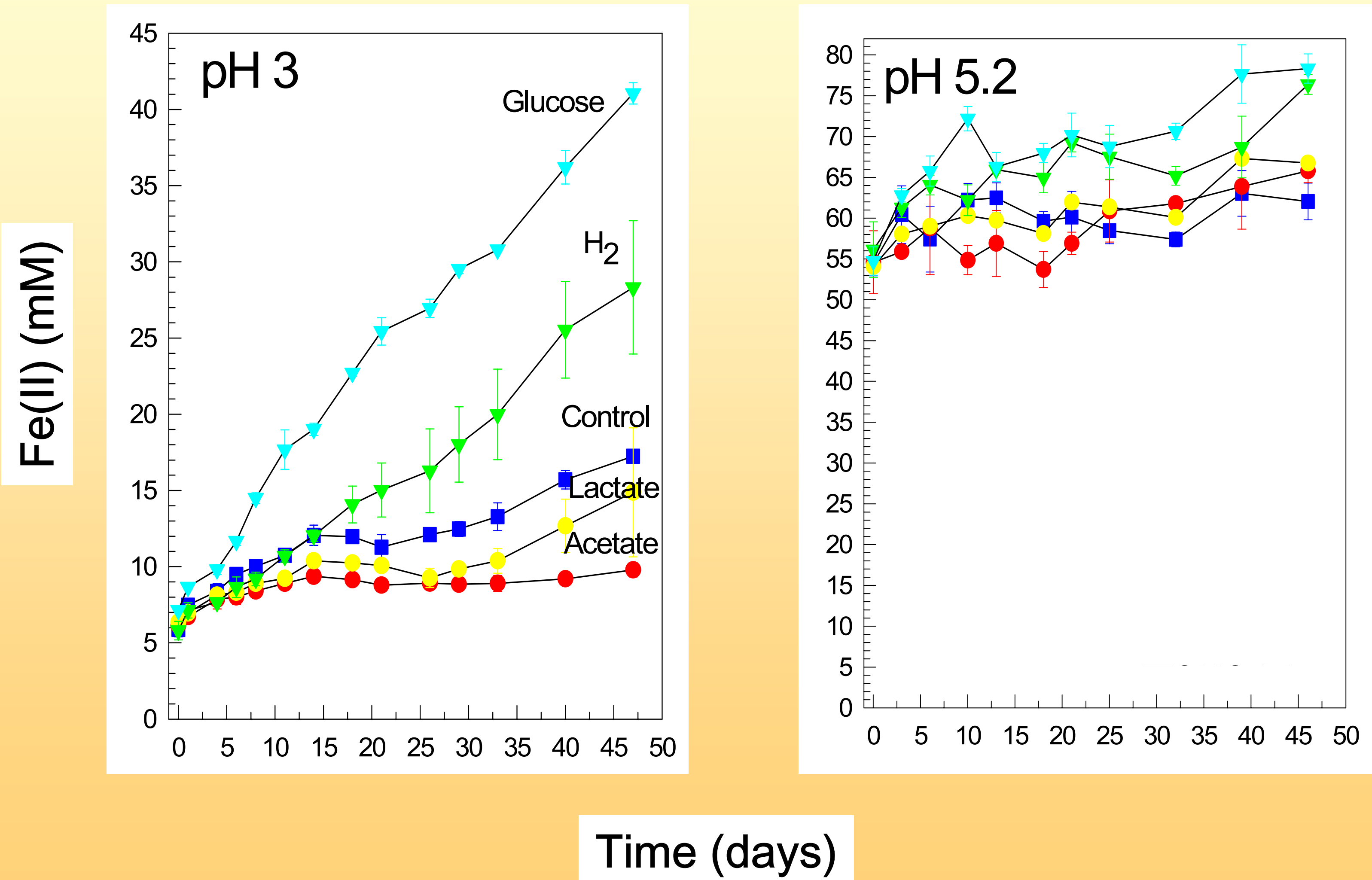
Microbial Diversity at pH 3 and pH 5 Zones





Very few clones were related to known Fe(III)-reducing bacteria!

What stimulates the reduction of Fe(III)?

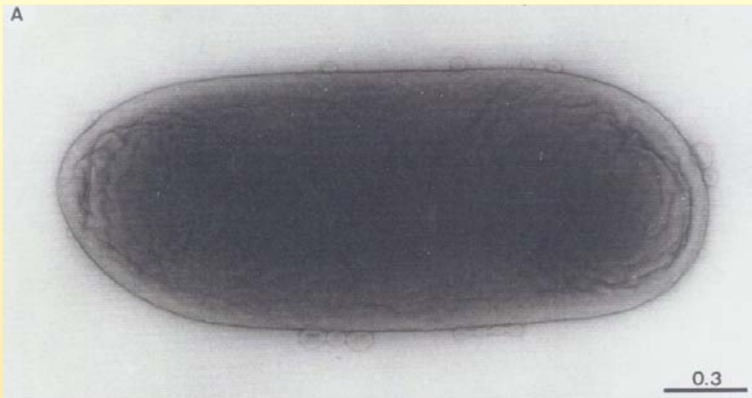


MPN Values of the Fe(III)-reducing Microbiota

Fe(III) reducer	Fe(III) source	pH	MPN g (wet wt. sediment)$^{-1}$ pH 3 Zone I	MPN g (wet wt. sediment)$^{-1}$ pH 5 Zone III
S^0-utilizing	schwertmannite	3.0	2.0×10^6	2.0×10^4
S^0-utilizing	goethite	5.0	5.0×10^2	2.1×10^2
Glucose-utilizing	schwertmannite	3.0	5.0×10^6	5.0×10^6
Glucose-utilizing	goethite	5.0	7.0×10^2	2.0×10^3
Lactate-utilizing	schwertmannite	3.0	90	0
Lactate-utilizing	FeOOH	5.0	n.d.	2.3×10^4
EtOH-utilizing	FeOOH	5.0	n.d.	2.3×10^5
Total cell counts			9×10^8	5×10^8

[a] MPN dilutions were incubated in three replicates at 15°C for 6 months.

Isolation of *Acidiphilium cryptum* JF-5

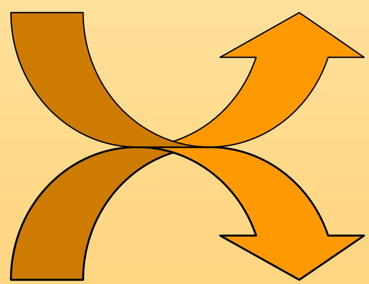


acidophilic, heterotrophic Fe(III) reducer (α-*Protoebacteria*)

facultative anaerobic, gram-negative rod

soluble and solid phase Fe(III) are reduced at pH 2.3 to 5

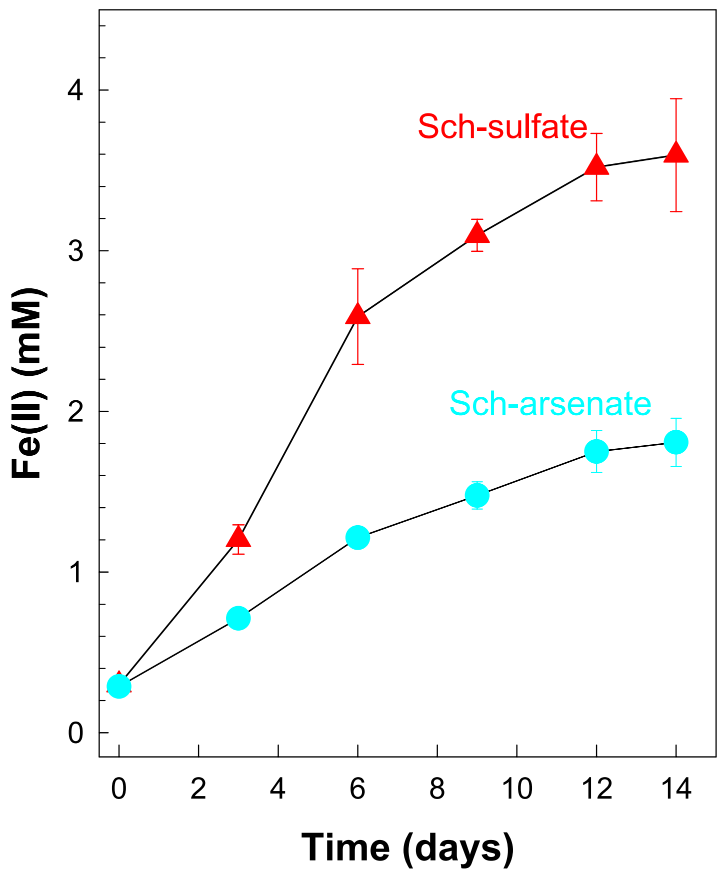
Fe(III) **Fe(II)**



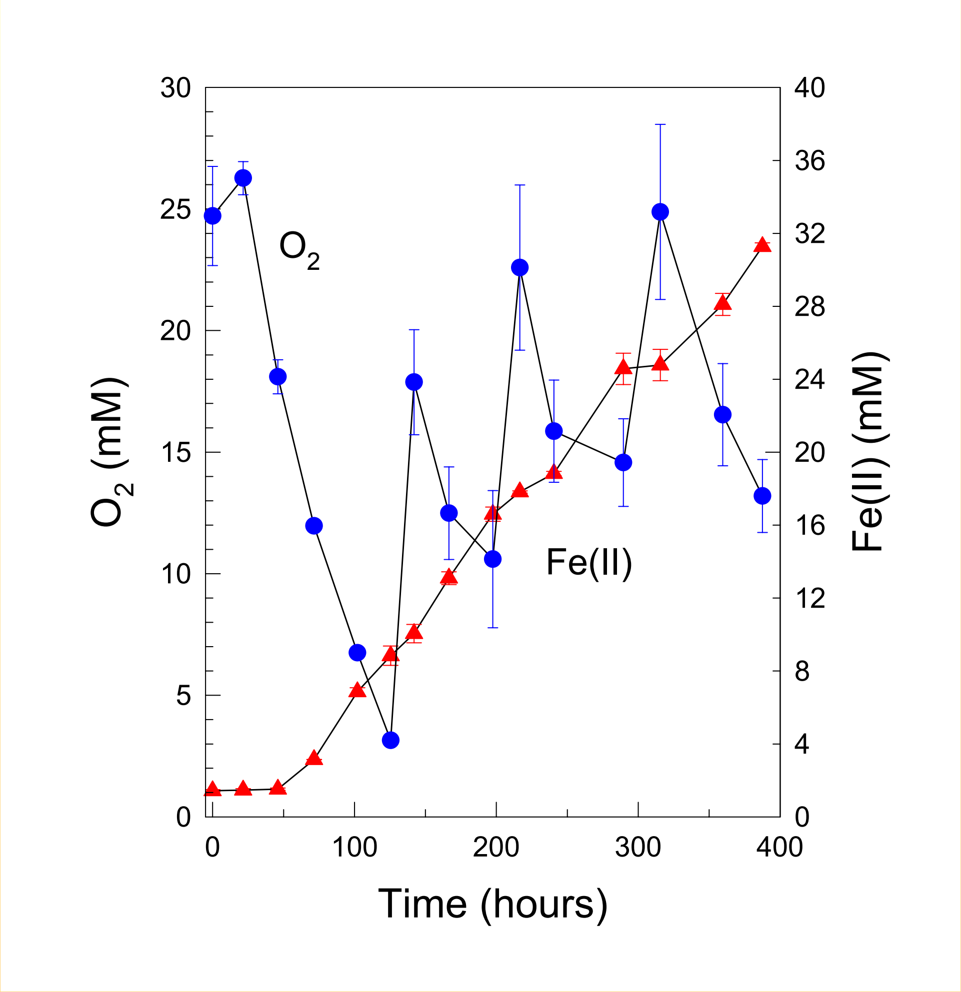
Glucose **CO_2**

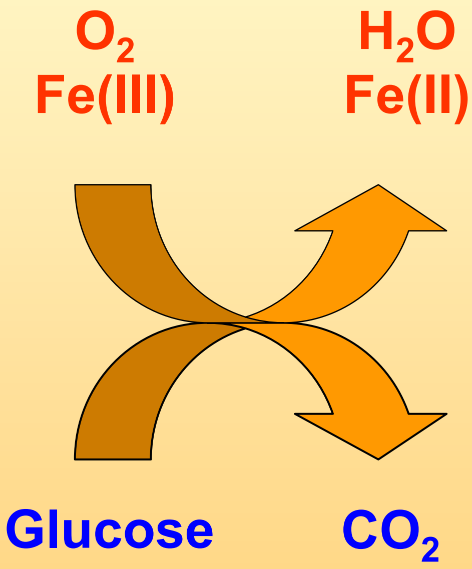
$$C_6H_{12}O_6 + 24\ Fe(III) + 24\ OH^- \rightarrow 6\ CO_2 + 24\ Fe(II) + 18\ H_2O$$

Effect of Incorporation of Arsenate on the Reductive Dissolution of Schwertmannite

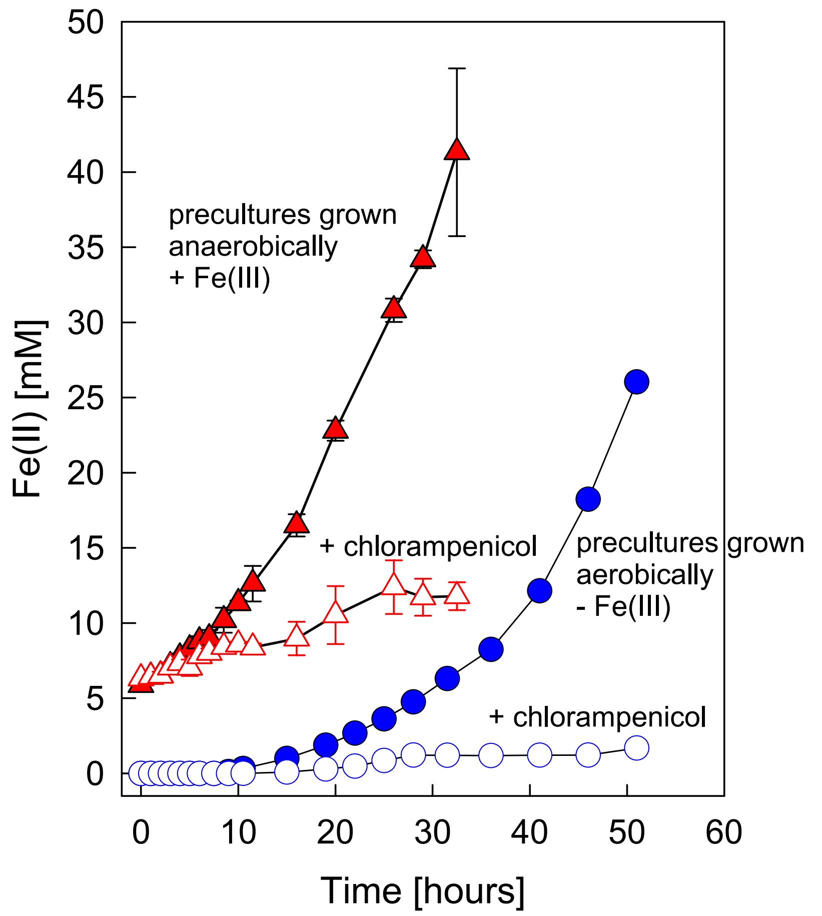


Co-Respiration of O_2 and Fe(III)





Are the enzymes involved in Fe(III) reduction constitutive?

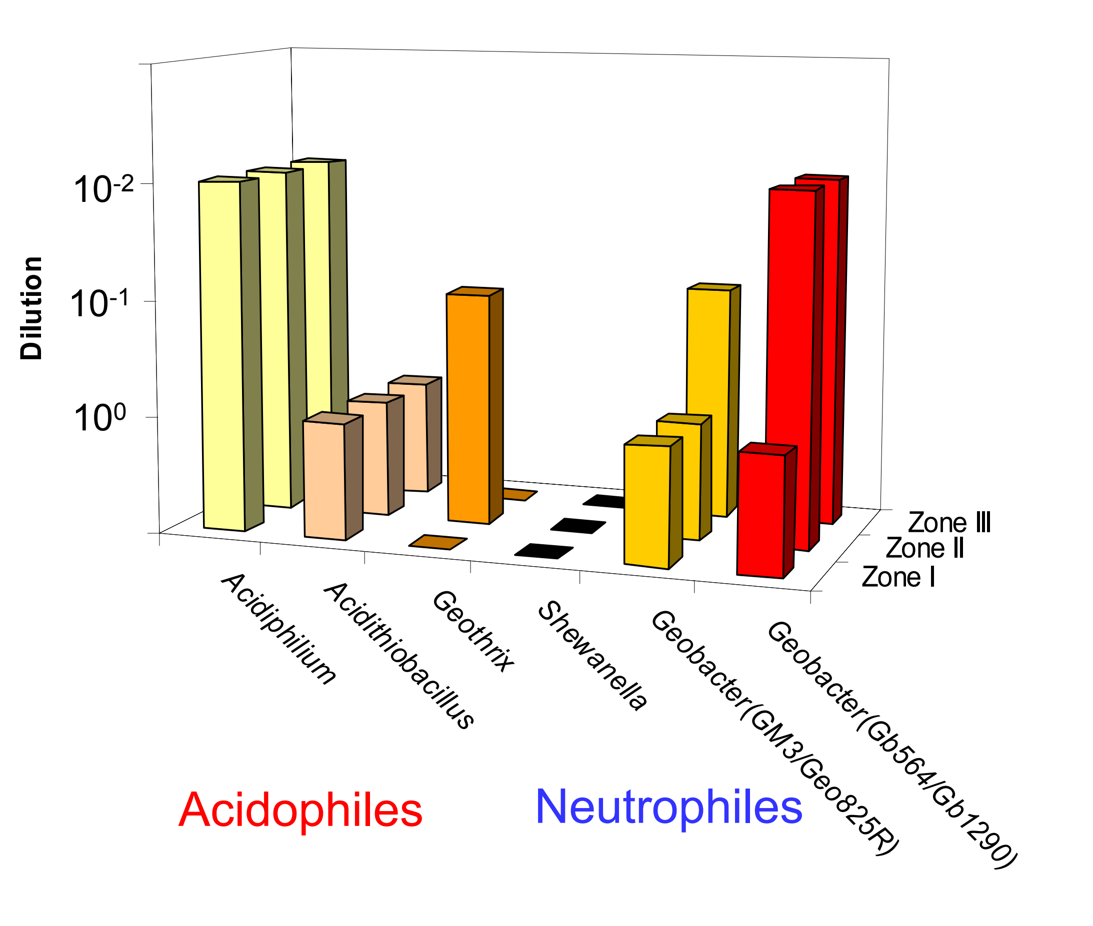


No!

What about other *Acidiphilium* species?

Species	Growth in the absence of oxygen (+ FE(III))	Rates of Fe(III) reduction in the presence of oxygen	Enzyme(s) involved in Fe(III) reduction	Source
Acidiphilium cryptum JF-5	yes	lower with increasing DO concentrations	inducible	this study
Acidiphilium SJH	poor	low rates at 20-40% DO constant at 40-60% DO	constitutive	Johnson and Bridge, 2002
Acidiphilium acidophilium	no	high rates at 20-40% DO low at 60-80% DO	inducible	Johnson and Bridge, 2002

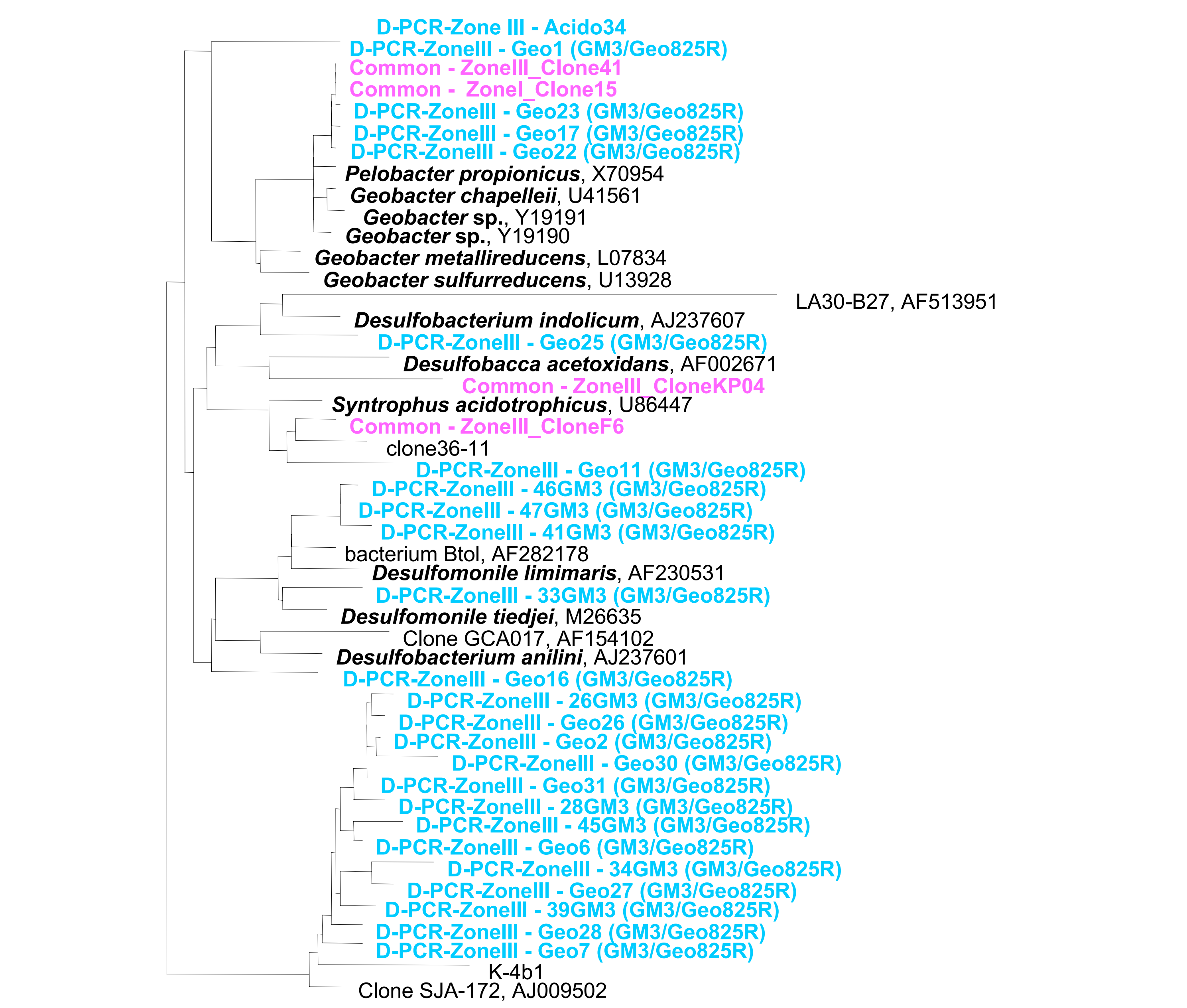
Use of Specific Primers for Fe(III) Reducers



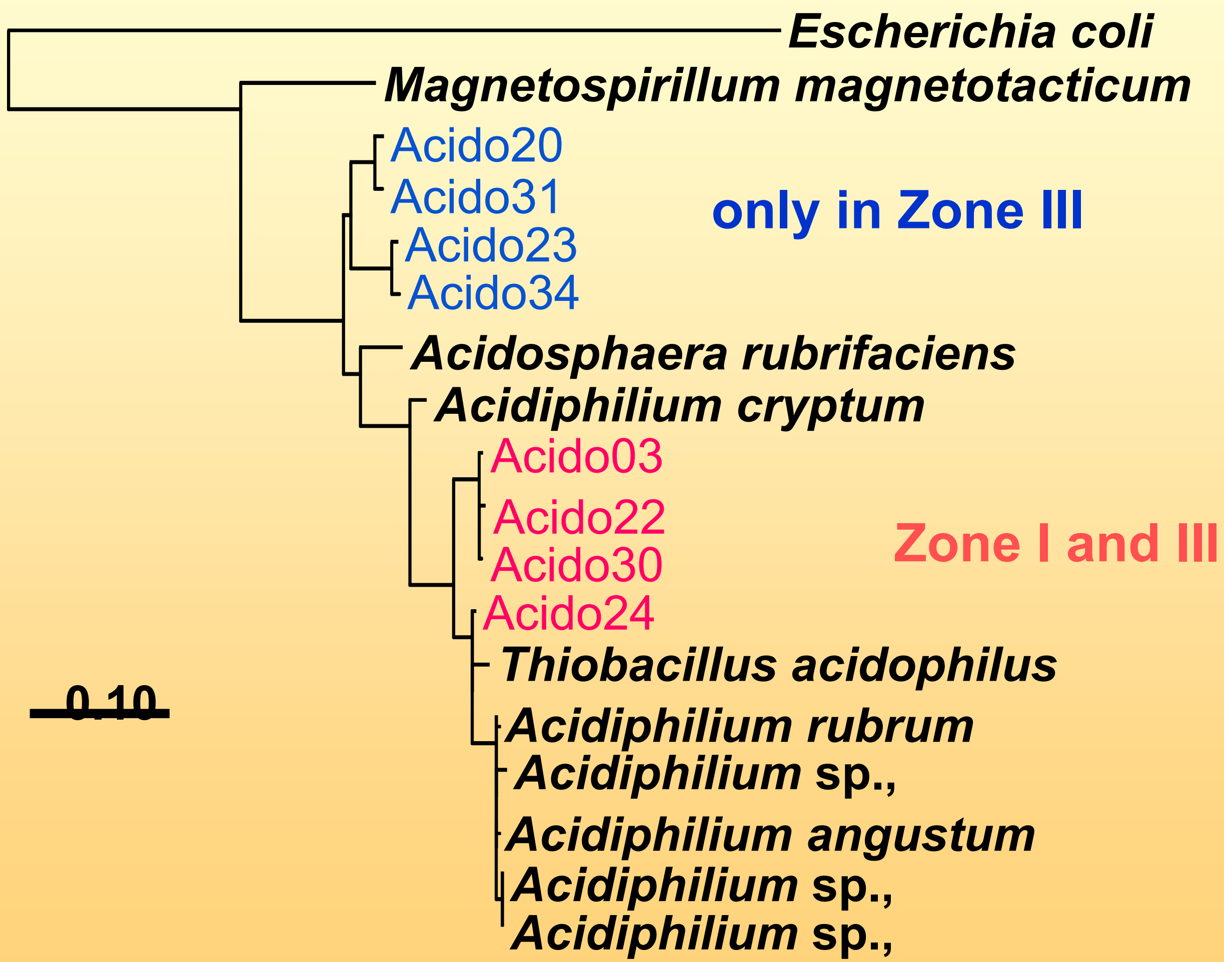
Sequences from the “Geobacter“ PCR product

Closest relative	No. of clones	Similarity (%)	
Geobacter grbcium strain TACP-5	**1**	90	***Geobactereraceae***
Geobacter sp. Strain CdA-2	**3**	93-97	
Bacterial species (clone kb2426)	**1**	92	***Acidobacterium***
Uncultured eubacterium WD247	**1**	96	
Uncultured eubacterium	**1**	90	***Caldothrix***
Uncultured bacterium clone FW43	**1**	91	***Actinomyces***
Uncultured bacterium clone 36-11	**1**	89	***Syntrophus***
Uncultured bacterium clone GOUTA19	**1**	93	***Magnetobacterium***
Syntrophobacter wolinii	**1**	91	***Syntrophobacter***
Bacterium K-4b1	**9**	93-96	
Uncultured bacterium SJA-172	**3**	94-95	
Uncultured bacterium clone LA30-B27	**1**	91	***Desulfobacterium***
Uncultured bacterium clone GCA017	**1**	93	
Desulfomonile limimaris	**1**	91	***Desulfomonile***
Uncultured bacterium Btol	**3**	93-94	
Angiococcus disciformis	**1**	89	
Uncultured bacterium RCP1-10	**3**	95-98	***γ-Proteobacteria***

Phylogenetic Tree of the "*Geobacter* " Clones



Phylogenetic Tree of the *Acidiphilium* Clones



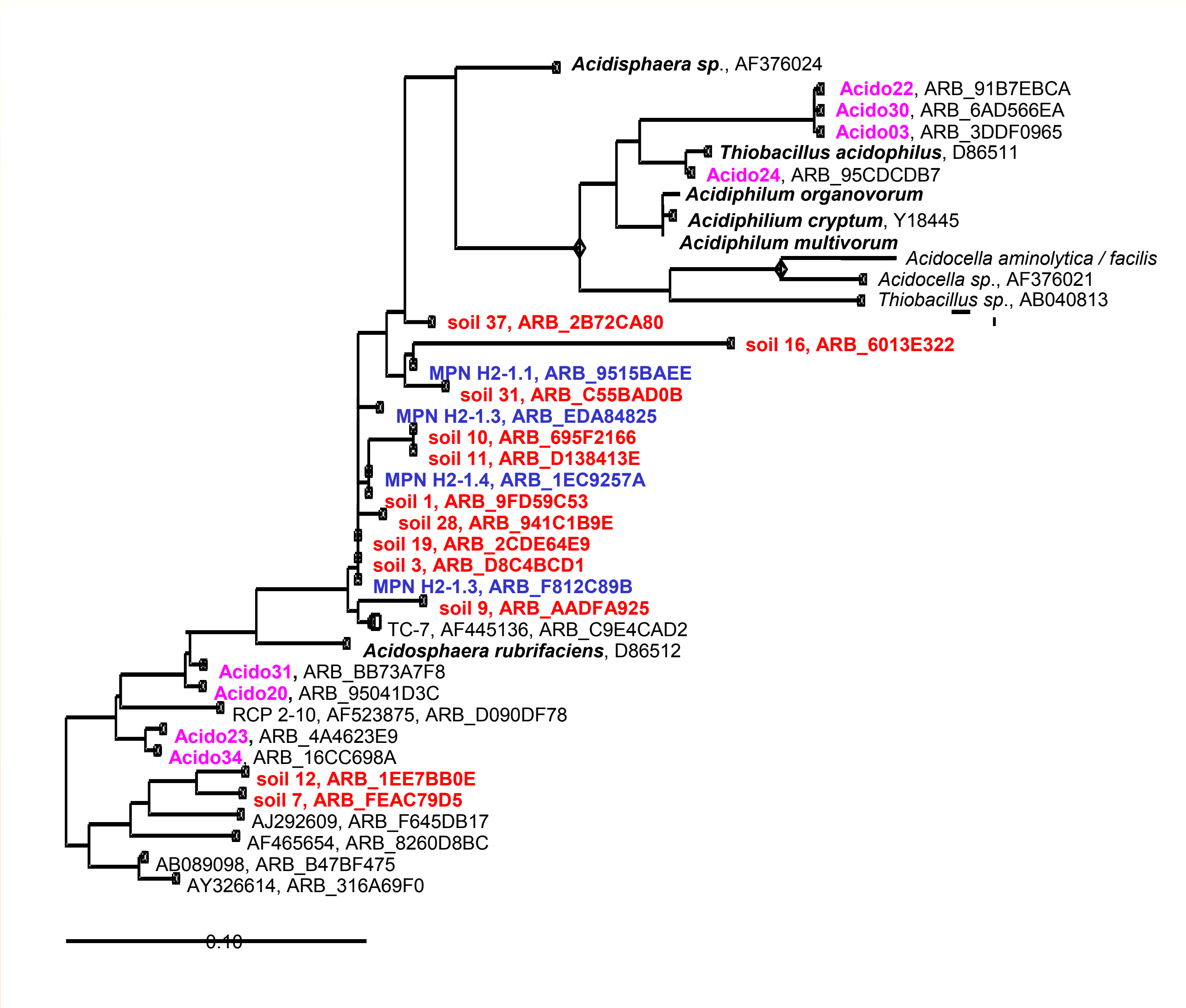
What about other pH 5 Fe(III)-reducing environments?

PCR Products for Potential Fe(III) Reducers at pH 5

Table 1. PCR-products obtained with specific primers for potential Fe(III)-reducing microbes

DNA-extract	Primer set specific for				
	Geobacter	Geothrix	Shewanella	Acidiphilium	Acidi-thiobacillus
peat	+	+	-	+	-
MPN H_2 10^{-1}	+	+	-	+	-
MPN H_2 10^{-3}	+	-	-	-	-
MPN acetate 10^{-1}	+	+	-	+	-
MPN acetate 10^{-3}	+	-	-	+	-
MPN glucose 10^{-1}	+	+	-	+	-

Phylogenetic Tree of the "*Acidiphilium* " Clones from Peat



Summary

- Higher microbial diversity in pH 5 than in pH 3 environments.

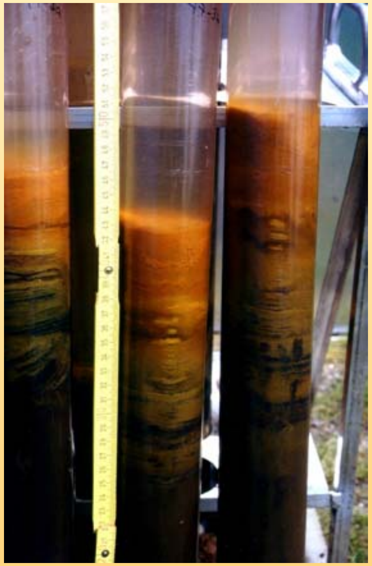
- *Acidiphilium* species appear to be involved in the reduction of Fe(III) in acidic environments.

- *Acidiphilium* species differ in their physiological capabilities and in their biochemistry from known neutrophilic Fe(III) reducers.

Acknowledgements to:

University of Bayreuth
Dept Ecol.Microbiology

Harold L. Drake
Tanja Trinkwalter
Kathrin Walter
Ursula Roth
Marco Blöthe
Sonja Trenz
Daria Schulz



University of Bayreuth
Limnol. Research Station

Ariane Peine
Andrea Tritschler
Simona Regenspurg
Christian Blodau
Winfrid Schulz-Gade
Stefan Peiffer