ORNL/CP-97844

CONF-980817--

Extremophilic iron-reducing bacteria: Their implications for possible life in extraterrestrial environments

Jizhong Zhou[#], Shi V. Liu, Chuanlun Zhang, Anthony V. Palumbo, and Tommy J. Phelps
Environmental Sciences Division, Oak Ridge National Laboratory*, Oak Ridge, TN 37831-6038.

[#]Correspondence: Environmental Sciences Division, POB 2008, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6038. 423-576-7544; E-mail: zhouj@ornl.gov.

An invited paper to

The Third Symposium of Chinese Young Scientists

August 20-22, 1998

Beijing, P. R. China

*Oak Ridge National Laboratory is managed by Lockheed Martin Energy Research Corporation for The U.S. Department of Energy.

DISCLAIMER

DISCLAIMER

Portions of this document may be illegible electronic image products. Images are produced from the best available original document.

Extremophilic iron-reducing bacteria: Their implications for possible life in extraterrestrial environments

Jizhong Zhou[#], Shi V. Liu, Chuanlun Zhang, Anthony V. Palumbo, and Tommy J. Phelps
(Environmental Sciences Division, Oak Ridge National Laboratory*, Oak Ridge, TN 37831-6038).

[#]Correspondence: Environmental Sciences Division, POB 2008, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6038. 423-576-7544; E-mail: zhouj@ornl.gov.

1. Abstract

Iron reduction is believed to be an early form of respiration and iron-reducing bacteria might have evolved very early on Earth. To support this hypothesis, we began to search for both thermophilic and psychophilic iron-reducing bacteria because iron-reducing capacity may be a widely distributed trait if ancestral microorganisms include extremophilic iron-reducing bacteria. To date, we have obtained thermophilic Fe(III)-reducing and magnetite-forming enrichment cultures from geologically and hydrologically isolated, millions of years-old deep terrestrial subsurface samples. Three dominant bacteria were identified based on 16S ribosomal RNA gene sequences. Phylogenetical analysis indicated that these bacteria were closely related to *Thermoanaerobacter ethanoliticus*. Two pure thermophilic iron-reducing bacteria have been isolated and characterized from these enrichments, they also are able to degrade cellulose and xylan. Geological evidence indicated that these bacteria were separated from modern organisms for about 200 million years, and they are the oldest isolated bacteria available now. Evolutionary sequence analysis showed that the 16S rRNA genes evolved extremely slowly in these bacteria. In addition, we have obtained about 30 psychrophilic iron-reducing bacteria in samples from Siberia and Alaska permafrost soils, Pacific marine sediments and Hawaii deep sea water. These bacteria were also able to reduce other heavy metals. The isolation of both thermophilic and psychrophilic iron-reducing bacteria from surface and subsurface environments has significant implications for microbial evolution and for studying the origin of life in extraterrestrial environments.

Key words: Iron reducing bacteria, metal reduction, exptremophiles, thermophiles, psychrophiles, molecular evolution, Astrobiology, Exobiology

2. Introduction

Dissimilatory Fe(III) reduction not only greatly influences the biogeochememical cycles of carbon and many metals but also has very important implications on the evolution of microbial life (Lovley 1991; Nealson and Saffarini 1994). Microorganisms capable of coupling organic compound oxidation with Fe(III) reduction are phylogenetically diverse, including members of gamma and delta *Proteobacteria*, Gram-positive bacteria and Flexistipes bacteria[1-6].

Geological evidence suggested that dissimilatory Fe(III) reduction could be an early form of microbial respiration[1]. It has been hypothesized that the primitive earth was hotter than it is today[7-8], and thermophily might be a characteristic associated with Fe(III) reduction. While bacterial reduction of Fe(III) under mesophilic condition is well-documented[1,2,9], microbial reduction under thermophilic conditions has not, and only a few phylogenetically distinctly thermophilic iron-reducing bacteria have been isolated and characterized[4,5,6]. On the other hand, no psychrophilic dissimilatory iron-reducing bacteria

have been reported, although many psychrophiles have been isolated[10]. Since more than 80% of the Earth's biosphere is permanently cold and undergoes significant temperature fluctuations, cold adaptation might also have occurred in iron-reducing microorganisms.

Recently, we reported isolation of thermophilic enrichment cultures capable of reducing Fe(III)-oxyhydroxide to magnetic iron oxides[11]. As an extension of this study, we continue to isolate thermophilic iron-reducing bacteria from these enrichment cultures. Meanwhile, we are expanding our studies to include psychrophiles which are capable of dissimilatory Fe(III) reduction. This paper summarizes research results from both directions of our investigations.

3. Magnetite production by thermophilic iron-reducing bacteria

To isolate thermophilic iron-reducing bacteria, samples were obtained from two geologically and hydrologically separated sedimentary deep subsurfaces, the Triassic-age Taylorsville Basin in Virginia and the Cretaceous-age Piceance Basin in Colorado, USA. Geological evidence suggested that the deep basins were separated from the surface for millions of years. The current temperature of the sampling depth (860-2800 m below land surface) ranges from 42-85 °C, and fluid pressure ranges from 30 to 35 MPa. The sampling processes and quality control were described elsewhere[11,12].

The samples were incubated at 60 °C in mineral media containing amorphous Fe(III) oxyhydroxide as an electron acceptor, and hydrogen, formate, acetate, lactate or pyruvate as the electron donors. After 2 weeks, black magnetic precipitates were observed in all of the treatments except the treatments with hydrogen and pyruvate as electron donors for the sedimentary rocks from Taylorsville Basin[11]. Scanning electron micrographs showed that iron minerals were formed as aggregates outside the bacterial cells (Fig. 1). The mineralogical compositions were analyzed by X-ray diffraction patterns at different time intervals of incubation[13]. Initially amorphous Fe(III)-oxyhydroxide predominated, and after about 3 days, magnetite dominated in the X-ray diffraction patterns (Fig. 2). No magnetite was observed in the presence of metabolic inhibitors[11], suggesting that the magnetite formation is due to biological processes rather than abiotic processes[11].

4. Molecular characterization of the thermophilic iron-reducing enrichment cultures

To further characterize the thermophilic iron-reducing enrichment cultures, a 16S ribosomal RNA (rRNA) gene-based molecular approach was used. The DNA was isolated from the three enrichment cultures with hydrogen, acetate and pyruvate as the electron donors from the Piceance Basin in Colorado as described previously[14]. The 16S rRNA genes were amplified by polymerase chain reaction (PCR), and the amplified products were cloned into plasmid vectors[15]. The 16S rRNA gene inserts were then directly amplified and differentiated using 4 tetrameric restriction enzymes. The restriction fragment length polymorphism (RFLP) patterns were compared by computer programs, GelComp, and the unique 16S rRNA gene clones were sequenced as described previously[16]. For convenience, each unique RFLP pattern was designated as an operational taxonomic unit (OTU).

A total of 36, 30, and 24 OTUs were observed for hydrogen-, acetate-, and pyruvate-grown cultures, respectively. Among them, three dominant OTUs were identified and together they constituted about 65% of the total 16S rDNA clones. The hydrogen- and pyruvate -grown cultures shared all three dominant OTUs but the acetate-grown culture lacked one of the three. The clone diversity appears to be higher in hydrogen-grown culture than pyruvate- and acetate-grown cultures and more OTUs were shared between hydrogen- and pyruvate-grown cultures than acetate-grown-culture.

Phylogenetic analysis of the selected 21 clones revealed the presence of three clusters of 16S rRNA gene sequences (Fig. 3). All three clusters were affiliated with the *Acetogenium* subgroup within the *Syntrophomonas* group of the Clostridia subphylum of Gram-positive bacteria, and closely related to *Thermoanaerobacter ethanolicus*. While two of the three dominant OTUs were about 97% similar to *Thermoanaerobacter ethanolicus* based on the 5' end sequences, the other dominant OTU was about 87% similar to this strain based on the 5' end sequences and about 95% similar based the full sequences. These results suggest the existence of a novel group of thermophilic iron-reducing bacteria. These bacteria are also distinctly different from all known mesophilic iron-reducing bacteria[3]. The results of this study support the claim that metal reduction may be a characteristic that is widespread in the domain of Bacteria[3]. The existence of such phylogenetically distinct thermophilic iron-reducing bacteria in the geologically isolated, millions of years-old deep subsurface samples also supports the hypothesis that dissimilatory iron-reducing bacteria may be the early forms of microbial respiration[1].

One important interesting question is which bacteria are potentially responsible for magnetite production. We hypothesized that the major (group of) Fe(III)-reducing bacteria should be present in all three types of cultures and the autotrophic Fe(III)-reducing bacteria should be at least present in the hydrogen-grown cultures. The molecular analyses of the microbial community structure of the three types of enrichment cultures yielded evidence to support this hypothesis. Despite the difference in the OTU composition, all three types of Fe(III)-reducing enrichment cultures shared two of the three dominant OTUs. These two OTUs, represented by clones P-3 and P-9, are most likely candidates for H_2 oxidation-coupled Fe(III) reduction. To make a further differentiation, we tested the capability of autotrophic Fe(III) reduction by a pure culture (TOR-39) isolated from thermophilic Fe(III)-reducing enrichment culture in our study of another deep subsurface site because TOR-39 is closely related to *Thermoanaerobacter ethanolicus* (98.9% sequence similarity) as is the P-9 clone (97.2% sequence similarity). The results showed that TOR-39 was incapable of autotrophic Fe(III) reduction (data not shown). Therefore, we speculate that the dominant OTU represented by the clone P-3 was most likely responsible for H_2 oxidation-coupled Fe(III) reduction and magnetite production. However, further experiments such as *in situ* rRNA hybridization are needed for testing this hypothesis.

5. Molecular evolution rates of the ancient thermophilic iron-reducing bacteria

Two iron-reducing bacteria were isolated from the enrichment cultures. These isolates were also able to degrade xylan or cellulose. Nearly full 16S rRNA genes from these two isolates were sequenced. Sequence analysis indicated that these two strains were very closely related to each other with the similarity of 99.5%. Phylogenetic analysis showed that they were clustered with *Thermoanaerobacter enthonoliticus* with the similarity of 97%. Geological evidence indicated that these bacteria were separated from the modern organisms for about 200 million years, and to our knowledge, they are the oldest-isolated viable bacteria now available.

We have compared the "absolute evolutionary rates" of 16S rRNA genes among these isolates. The absolute molecular evolution rates were estimated as $2.8\text{-}4.2\times10^{-11}$ substitutions/year/site. This estimate is significantly lower than the other estimations based on 16S rRNA genes, e.g. $1.8\text{-}2.4\times10^{-9}$ for ancient amber bacteria[17], $1.8\text{-}2.4\times10^{-9}$ for eubacteria[18], and $0.1\text{-}0.4\times10^{-9}$ for aphid bacterial symbionts. These results suggested the 16S rRNA genes evolved extremely slowly in these bacteria.

6. Psychrophilic iron-reducing bacteria

We have obtained samples from a variety of cold environments such as Siberia and Alaska permafrost soils, deep marine sediments and Hawaii deep sea water. These samples were incubated under iron-reducing conditions at 5-25 °C using ferric citrate (10 mm) as an electron acceptor and organic acids (10 mm) or hydrogen (80% balanced with 20% CO_2) as an electron donor (Table 1). All the enrichment cultures used molecular hydrogen and pyruvate for iron reduction. Growth with acetate was fastest in Pacific marine sediments enrichments, slower in Alaskan enrichments, and was absent in Hawaii enrichments (Table 1). This suggests that microbial community associated with iron reduction was different among these sampling locations.

The rates of iron-reduction by psychrophilic bacteria were different than those of mesophilic and thermophilic iron-reducing bacteria. Usually a longer period of slow reduction preceded the log phase than mesophilic species. Once reaching the log phase psychrophilic bacteria reduced Fe(III) as fast as mesophilic species such as *Shewanella alga* BrY. In general, however, the lower the temperature, the longer the slow period of growth. For example, it took less than five days for psychrophilic bacteria to reach the stationary phase growth at 10°C, but longer than 10 days at 5°C.

All psychrophilic iron-reducing bacteria could form siderite when reducing soluble ferric citrate. This is probably due to oversaturation with respect to Fe(II) and carbonate. The bacterial cells, on the other hand, may have facilitated the precipitation by providing nucleation sites and lowering the energy barrier for mineral precipitation.

Enrichment cultures from Alaskan permafrost also reduced other metals including Co(III) and U(VI), but not Cr (VI), suggesting that chromium may be toxic to the bacterial activity at the concentration tested.

Table 1. Summary of psychrophiles from various geological environments and their capability of metal reduction

Sample location	No. of enrichments	Geological age (year)	Lithology and chemistry	Metabolic capability	Tested growth temperature (°C)	Metals reduced
Siberian permafrost	5	10K-4M	clay, sand	H_2 pyruvate	15	Fe(III)
Alaskan permafrost	1	6 M	clay	H_2 pyruvate acetate lactate	5-25	Fe(III), U(VI), Co(III)
Hawaii deep sea water	3	Modern	water rich in iron, pH 7-7.8	H_2 pyruvate	15	Fe(III)

Pacific sediments	20	Modern	clay, fine sand	H_2 pyruvate acetate lactate formate	10	Fe(III)

7. Implications for possible life at extraterrestrial environments

The existence of life on other planets beyond Earth has intrigued scientists for centuries. At the present time, the best place for extraterrestrial life in solar system are thought to be Mars and the of Jupiter. Recently, several lines of evidence were given to suggest the presence of biogenic activity on Mars[19]. Among the important evidence is the occurrence of magnetite and iron sulfide particles, which could have resulted from oxidation and reduction reactions known to be important to terrestrial microbial systems. However, interpretations of this evidence are inconclusive because the magnetite minerals can be explained by either biological or abiotic processes. If they were of biological origins, the thermophilic iron-reducing bacteria would be responsible for such activities because, similar to the early earth, early Mars was hot. Although magnetite formation was observed in terrestrial mesophilic microorganisms, little is known about the magnetite production by thermophilic iron-reducing bacteria. Thus our studies on thermophilic iron-reducing and magnetite-producing bacteria add a strong dimension of using terrestrial analogs for studying the microbial life on Mars.

The surface of present mars is very cold (–60 °C) and permafrost conditions may prevail to a considerable depth. If life exists in present Mars, psychrophiles may be like found at depth. This is supported by our finding of psychrophiles in deep Siberian permafrost, which are able to survive and grow at low temperatures for up to 4 millions of years (Table 1). Since the interpretation of the potential biogenic activity on Mars is based largely on what we know about the life processes on Earth, the studies on the diversity, cold adaptation, mechanisms and life limits of terrestrial psychrophiles will be of great value in explaining the possible evidences in Marian samples. Thus our studies on psychrophilic iron-reducing bacteria will provide model organisms for studying cold adaptation and mechanisms.

Possibilities of life on Mars, if ever present, may be explained by: (1) independently evolved life; (2) ancient inoculation of life on that foreign body; (3) mission-derived contamination, and (4) derivation from abiotic processes. Mutipleline evidence in terms of mineralogical biomarkers, and biomarkers associated with life evolutionary processes and life responses and adaptations to stressors are needed to differentiate these possibilities. Martian microorganisms may be detected by molecular methods used to analyze for terrestrial genetic machinery. However, the evolutionary processes of Martian microorganisms could be different from those of terrestrial microorganisms because the planetary evolution processes of Mars is different from those of Earth. Thus the selection pressure on microorganism evolution on Mars could be distinctly different from that on Earth. Such differences could be detected and used as evidence for the existence of life on Mars by thorough understanding the evolutionary history, patterns, rates and mechanisms of terrestrial microorganisms. For example, if microorganisms found in Martian samples do not fit into the evolutionary processes of terrestrial microorganisms and their genetic machinery and the genes involved in various metabolic processes are distinct from those in terrestrial microorganisms, they are most likely from independent origins. If microorganisms found in Martian samples have no or little divergence from terrestrial microorganisms, they are most likely from mission-derived contamination. If the microorganisms found in Martian samples have commonality in genetic machinery (e.g.

same nucleic acids, amino acids, RNA and DNA), and similar genes for different metabolic processes with terrestrial microorganisms, but their phylogeny is not consistent with those of the known taxa, then they are most likely from ancient inoculation and have a common origin with Earth-derived life. However, reliable estimation of divergence time for different terrestrial microorganisms is difficult to be obtained.

The molecular clock hypothesis[20], which assumes that homologous genes in different species should evolve at similar rates, make the quantitative reconstructions of historical events possible. Numerous studies have been conducted to determine the divergence times of different groups of organisms based on molecular data[21-25]. Nonetheless, the divergence times of major groups of organisms, especially for procaryotes, have remained elusive. One of the main reasons is that calibration of molecular clocks in procaryotes is extremely difficult because of the lack of reliable fossil records for microorganisms. Since the separation times of these bacteria from modern organisms are known, and cover up to 5% of the life history on Earth, they are extremely valuable for calibrating molecular clocks. Thus, the ancient thermophilic and psychrophilic iron-reducing bacteria we obtained will be extremely useful in understanding life evolutionary processes and in studying Astrobiology.

8. Acknowledgments

This project was supported by the U. S. Department of Energy under funding to Oak Ridge National Laboratory, which is managed by Lockheed Martin Energy Research Corp. for DOE under contract number DE-AC05-96OR22464. This research was also supported in part by an appointment (J.Z. Zhou) to the Alexander Hollaender Distinguished Postdoctoral Fellowship Program sponsored by the U.S. Department of Energy, Office of Health and Environmental Research, and administered by the Oak Ridge Institute for Science and Education (ORISE), the ORNL postdoctoral Research Associates Program administered jointly ORNL and ORISE (S. V. Liu and C. Zhang), the ORNL Laboratory Directed Research and Development Program.

9. References

1. Lovley, D. R. 1991. Dissimilatory Fe(III) and Mn(III) reduction. Microbiol. Rev. 55: 259-287.
2. Nealson, K. H., and D. Saffarini. 1994. Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. Annu. Rev. Microbiol. 48: 311-343.
3. Lonergan, D. J., H. L. Jenter, J. D. Coates, E. J. P. Phillips, T. M. Schmidt, and D. R. Lovley. 1996. Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. J. Bacteriol. 178:2402-2408.
4. Boone, D. R., Y. Liu, Z.-J. Zhao, D. L. Balkwill, G. R. Drake, T. O. Stevens, and H. C. Aldrich. 1995. Bacillus infernus sp. nov., an Fe(III)- and and Mn(IV)-reducing anaerobe from the deep terrestrial subsurface. Int. J. Syst. Bacteriol. 45: 441-448.
5. Greene, A. C., B. K. C. Patel, and A. J. Sheehy. 1997. Deferribacter thermophilus gen. nov., sp. nov., a novel thermophilic manganese- and iron-reducing bacterium isolated from a petroleum reservor. Int. J. Syst. Bacteriol. 47: 505-509.
6. Slobodkin, A., A.-L. Reysenbach, N. Strutz, M. Dreier, and J. Wiegel. 1997. Thermoterrabacterium ferrireducens gen. nov., sp. nov., a thermophilic anaerobic dissimilatory Fe(III)-reducing bacterium from a continental hot spring. Int. J. Syst. Bacteriol. 47: 541-547.
7. Ohmoto, H., and R. P. Felder. 1987. Bacterial activity in the warmer, sulphate-bearing Archean occeans. Nature 328:244-246.
8. Kasting,J.F. and T.P. Ackerman. 1986. Climatic consequences of very high carbon dioxide levels in the Earth's early atmosphere. Science 234: 1383-1385.

9. Lovley, D. R. 1993. Dissimilatory metal reduction. Annu. Rev. Microbiol. 47: 263-290.
10. Bowman, J. P., S. A. McCammon, M. V. Brown, D. S. Nichols, and T. A. McMeekin. 1997. Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl. Environ. Microbiol. 63: 3068-3078.
11. Liu, S., J.-Z. Zhou, C.-L. Zhang, D. R. Cole, M. Gajdarziska-Josifovska, and T. J. Phelps. 1997. Thermophilic Fe(III)-reducing bacteria from the deep subsurface: The evolutionary implications. Science: 277:1106-1109.
12. Phelps, T.J., Onstott, T.C., Balkwill, D., Fredrickson, J., Fletcher, M., Murphy, E., and Wobber, F.J. (1994) Work Plan for Analyses of the Taylorsville Triassic Basin Samples. 23p. U. S. Department of Energy, Office of Energy Research, Washington D. C.
13. Zhang C.-L., S. Liu, T. J. Phelps, D. R. Cole, J. Horita, S. M. Fortier, M. Elless, and J. W. Valley. 1997. Physiochemical, minerological, and isotopic characterization of magnetite-rcih iron oxides formed by thermophilic iron-reducing bacteria. Geochim. Cosmochim. Acta 61:4621-4632.
14. Zhou, J.-Z. M. A. Bruns, and J. M. Tiedje. 1996. DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. 62: 461-468.
15. Zhou, J.-Z, M. E. Davey, J. B. Figueras, E. Rivkina, D. Glichinsky, and J. M. Tiedje. 1997. Phylogenetic diversity of the bacterial community from a tundra soil sample. Microbiology 143:3913-3919
16. Zhou, J.-Z., M. R. Fries, J. Chee-Sanford, and J. M. Tiedje. 1995. Phylogenetic analysis of a new group of denitrifiers capable of anaerobic growth on toluene: Description of *Azoarcus tolulyticus* sp. nov. Int. J. Syst. Bacteriol. 45: 500-506.
17. Cano, R. J., and M. K. Borucki. 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old dominican amber. Science 268:1060-1064.
18. Ochman, H., and A. C. Wilson. 1987. Evolution in bacteria: evidence for a universal substitution rate in cellular genomes. J. Mol. Evol. 74-86.
19. McKay , D. S., E. K. Gibson Jr., K. L. Thomas-Keprta, H. Vali, C. S. Romanek, S. J. Clemett, X. D. F. Chillier, C. R. Maechling, and R. N. Zare. 1996. Search for past life on Mars: possible relic biogenic activity in Martian meteoreite ALH84001. Science 273:924-930.
20. Zuckerkandal, E., and L. Pauling. 1965. Evolutionary divergence and convergence in proteins. pp 97-166. In: Bryson V., Vogel, H (eds), Evolving genes and proteins. Academic Press, New York.
21. Doolittle, R. F., D.-F. Feng, S. Tsang, G. Cho, and E. Little. 1996. Determining divergence times of the major kingdoms of living organisms with a protein clock. Science 271:470-477.
22. Hedges, S. B., P. H. Parker, C. G. Sibley, and S. Kumar. 1996. Continental breakup and the ordinal diversification of birds and mammals. Nature 381:226-229.
23. Moran, N. A., C. D. von Dohlen, and P. Baumann. 1995. Faster evolutionary rates in endosymbiotic bacteria than in cospeciating insect hosts. J. Mol. Evol. 41: 727-731.
24. Wray, G. A., J. S. Levinton, L. H. Shapiro. 1996. Molecular evidence for deep Precambrian divergences among Metazoan phyla. Science 274:568-573.
25. Zhou, J.-Z., and A. Kleinhofs. 1996. Molecular evolution of nitrate reductase genes. J. Mol. Evol. 42: 432-442.

Authors: Jizhong Zhou, male, born on August 6, 1959. He obtained BS and MS from Hunan Agricultural University in 1981 and 1984 in plant protection and mathematical ecology, respectively. He was a research associate with the late Professor Shijun Ma in Eco-Environmental Research Center, Chinese Academy of Sciences from 1986 to 1988. He obtained his Ph.D in 1993 in molecular biology at Washington State University, Pullman, WA, USA. He is currently a staff scientist at Environmental Sciences Divisions, Oak Ridge National Laboratory. His expertise is in molecular biology, microbial ecology,

molecular evolution, DNA sequence analysis and theorectical ecology. His phone: 423-576-7544; Fax: 423-576-8646; E-mail: zhouj@ornl.gov.

This paper derives from a previous and current collaborative study with various scientists in different fields. This study was highlighted by CNN News in 1997, for the first discovery of thermophilic iron-reducing and magnetite-producing bacteria from deep subsurface environment. Shi V. Liu is a microbiologist and is currently at Department of Microbiology, Allegheny University of the Health Sciences. Chuanlun Zhang is a biogeochemist with strong background in microbiology and is currently at Department of Geology, University of Missouri. A. V. Palumbo and T. J. Phelps are staff scientists at Oak Ridge National Laboratory with specialty in microbial ecology and microbial physiology, respectively.

Figure legends

Fig. 1. Morphologies of bacteria and magnetic minerals. SEM images of samples prepared anaerobically with minimum disturbance.

Fig. 2. X-ray diffraction patterns for iron minerals preicipitated at 50 °C in enrichment cultures from Creaceous Piceane Basin, Colorado. A: Akaganeite; M: magnetite; S: siderite, H:Hematite.

Fig. 3. Phylogenetic relationships of the 21 phylotypes sequenced from the thermophilic iron-reducing communities. The tree was established by the maximum likelihood method based on the partial 16S rRNA gene sequences with *E. coli* as the outgroup.

ORNL-DWG 95M-7400R1

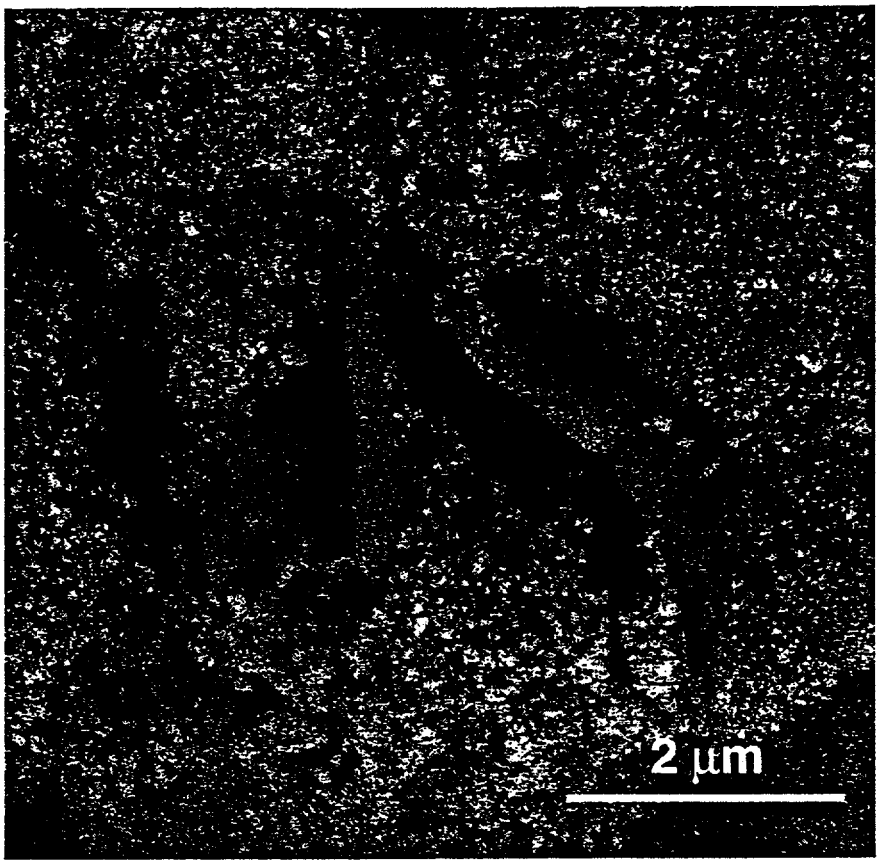

Fig 1

ORNL 98-686/rra

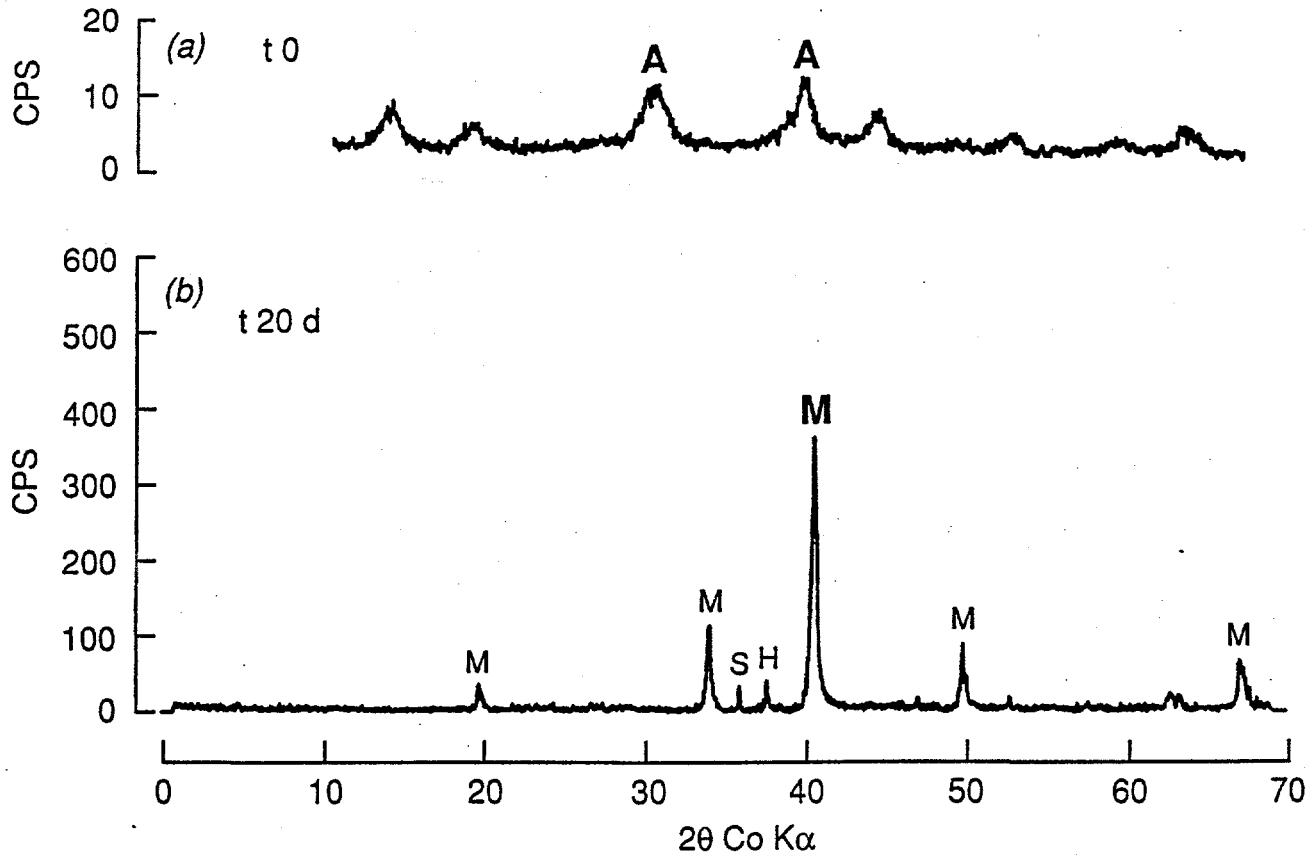

Fig. 3

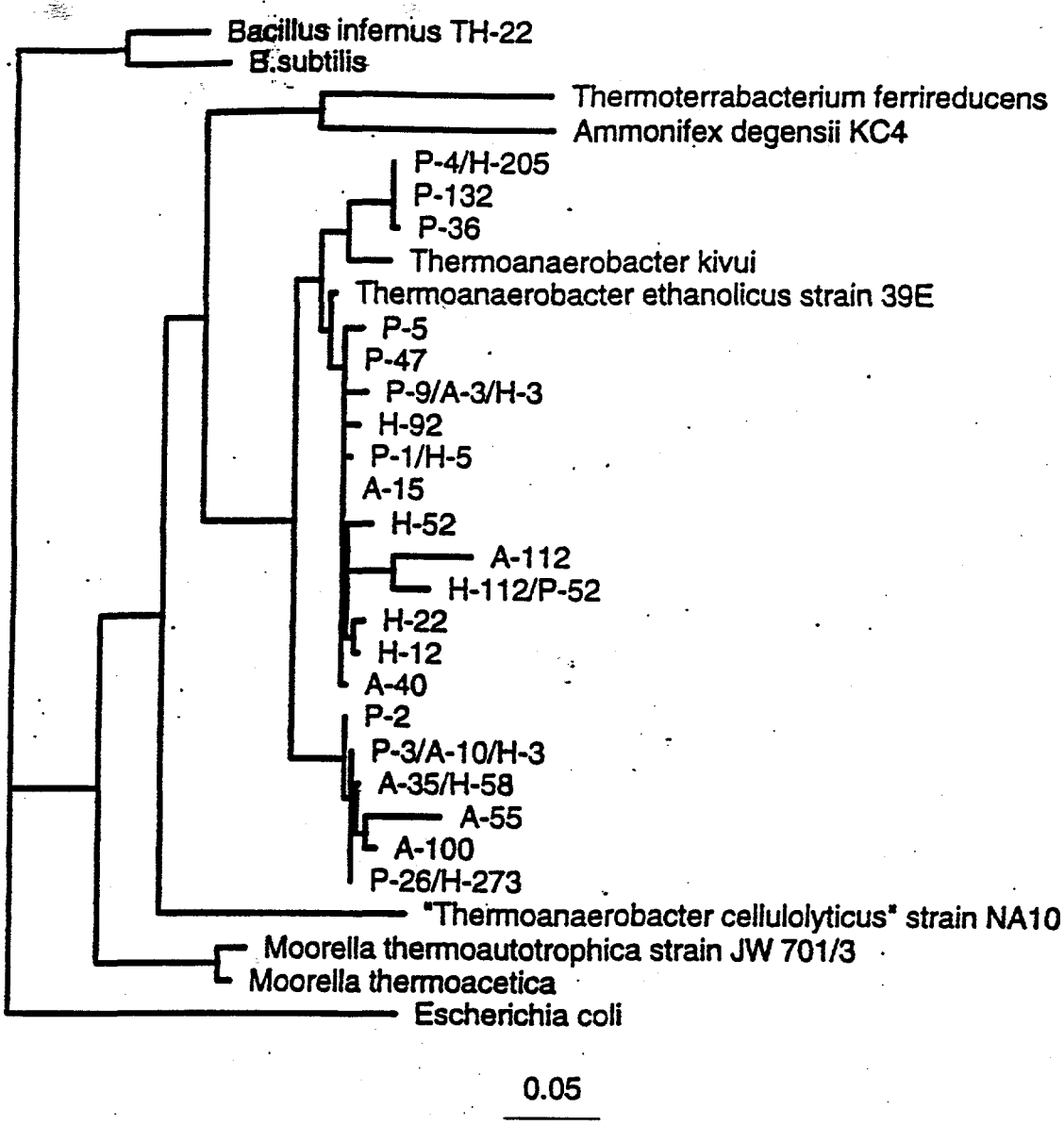
Bacillus infernus TH-22
B.subtilis
Thermoterrabacterium ferrireducens
Ammonifex degensii KC4
P-4/H-205
P-132
P-36
Thermoanaerobacter kivui
Thermoanaerobacter ethanolicus strain 39E
P-5
P-47
P-9/A-3/H-3
H-92
P-1/H-5
A-15
H-52
A-112
H-112/P-52
H-22
H-12
A-40
P-2
P-3/A-10/H-3
A-35/H-58
A-55
A-100
P-26/H-273
"Thermoanaerobacter cellulolyticus" strain NA10
Moorella thermoautotrophica strain JW 701/3
Moorella thermoacetica
Escherichia coli
0.05

Fig 3.