Appendix 1

1. Samples were obtained from a well-characterized reference outcrop of Columbia River Basalt, designated BWIP-EC URC.1 4-29-88, on Umtanum Ridge, Washington (Allen, C. C., Johnston, R. G., and Strope, M. B., 1985, NEED TITLE, RHO-SD-BWI-DP-053, U.S. Department of Energy, Richland, Washington). Weathered crusts were removed and samples were crushed in a ceramic-faced jaw mill. The pulverized material was sieved through nonmetallic screens to obtain a coarse size fraction. Microbial communities were harvested from reducing subsurface basaltic aquifers of the Columbia River Basalt group and grown in microcosms containing unweathered basalt chips and ground water as described by Stevens, T. O., McKinley, J. P., and Fredrickson, J. K., 1993, Bacteria associated with deep, alkaline anaerobic groundwaters in southeast Washington: Microbial Ecology, v. 25, p. 35. A sterile cartridge packed with unweathered basaltic sand was connected in parallel to the flow emerging from municipal well #4, City of Prosser, Washington. Ground water microorganisms attached to the basalt sand and formed dense biofilms during two weeks of flow. The cartridge was transported to the laboratory and the contents removed in an anaerobic chamber.

Microcosms consisted of sealed glass serum bottles containing 50 g of fresh basalt chips, from a reference outcrop of Columbia River Basalt, and 50 ml of anaerobic mineral salts solution (Stevens, T. O., and McKinley, J. P., 1995, Geochemically produced hydrogen supports microbial ecosystems in deep basalt aquifers: Science, v. 270, p. 450-454). Bottles were inoculated by adding 50 g of wet solids from the sand cartridge with its attached microbial biofilm. Basalt served as the sole electron donor and carbon dioxide as the sole electron acceptor. Microcosms were incubated in the dark at 30 °C for at least 8 weeks before examination. As described in Stevens, T. O., McKinley, J. P., and Fredrickson, J. K., 1993, Bacteria associated with deep, alkaline anaerobic groundwaters in southeast Washington: Microbial Ecology, v. 25, p. 35, this method inevitably results in a mixture of organisms derived from the aquifers and organisms derived from the well structure. However, the precise origin of individual cells is irrelevant to the current study. This source of organisms was selected because it was a ready source of microorganisms adapted or acclimated to a subsurface basalt rock habitat. For this study we

examined samples from one of the microcosms (P4 biomass), which included a diverse community of microorganisms, including both eubacteria and methanogenic archaea. This was determined by metabolic diversity assays, direct fluorescent observation, and direct extraction of nucleic acid sequences (Stevens, T. O., and McKinley, J. P., 1995, Geochemically produced hydrogen supports microbial ecosystems in deep basalt aquifers: Science, v. 270, p. 450-454; Goebel, B. M., Stevens, T. O., and Pace, N. R., 1997, Molecular analysis of a microcosm extablished form a deep subsurface aquifer, Abstracts, 97th General Meeting: American Society for Microbiology, p. 383).

2. For field emission gun scanning electron microscope analyses, most samples were coated with 2-5 nm of Au-Pd; one uncoated surface was examined at 2 kV. For analysis in the transmission electron microscope, critical pointdried, inoculated P4 biomass chips were sonicated in triple distilled water and the supernatant containing extracted microorganisms was transferred to transmission electron microscope copper grids with carbon-coated formvar films. These microorganisms were examined uncoated at 160 kV.

3. **Figure**

Size comparison of number of structures that have been interpreted as either microbial cells or their appendages. Approximate size range of Columbia River Basalt filaments is shown along with several features from martian meteorite ALH84001 (McKay, D. S., Gibson, E. K., Jr., Thomas-Keprta, K. L., Vali, H., Romanek, C. S., Clemett, S., Chillier, X. D. F., Maechling, C. R., and Zare, R. N., 1996, Search for past life on Mars: Possible relic biogenic activity in Martian meteorite ALH84001: Science, v. 273, p. 924). All ALH84001 features are within size field of Columbia River Basalt filaments. ALH84001 features are also close in size to submicrometer cells identified by various authors.

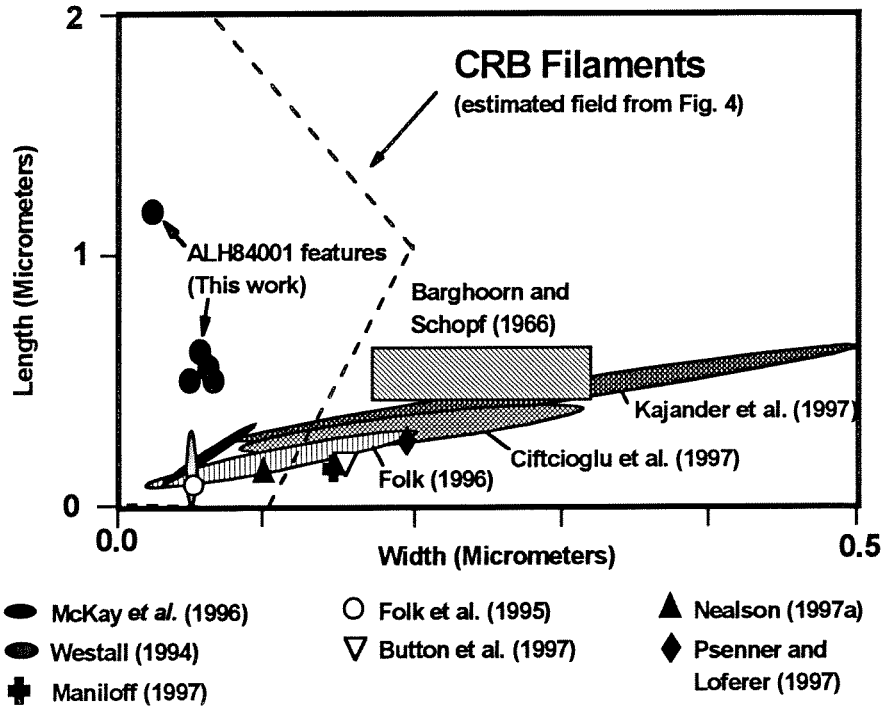

Figure References

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McKay, D. S., Gibson, E. K., Jr., Thomas-Keprta, K. L., Vali, H., Romanek, C. S., Clemett, S., Chillier, X. D. F., Maechling, C. R., and Zare, R. N., 1996, Search for past life on Mars: Possible relic biogenic activity in Martian meteorite ALH84001: Science, v. 273, p. 924.

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4. The ability of microorganisms to complex significant quantities of metal cations is well documented and facilitated by electrostatic interactions with anionic groups in the polymers of the cell walls (e.g., Mann, H., and Fyfe, W. S., 1989, Metal uptake and Fe-, Ti-oxide biomineralization by acidophilic microorganisms in mine-waste environments, Elliot Lake, Canada: Canadian Journal of Earth Sciences, v. 26, p. 2731-2735.; Beveridge, T.J. and Murray, R.G.E., 1980, Sites of metal deposition in the cell wall *of Bacillus subtilis*: Journal of Bacteriology, v. 141, p. 876-887; Beveridge, T.J. and Fyfe, W.S., 1985, Metal Fixation by bacterial cell walls: Canadian Journal of Earth Sciences, v. 22, p. 1892-1898; Beveridge, T.J. and Fyfe, W.S., 1985, Metal Fixation by bacterial cell walls: Canadian Journal of earth Sciences, v. 22, p. 1892-1898; Konhauser, K.O., Fyfe, W.S., Ferris, F.G., and Beveridge, T.J., 1993, Metal sorption and mineral precipitation by bacteria in two Amazonian river systems: Rio Solimões and Rio Negro, Brazil, Geology, V. 21, p. 1103-1106.). Mechanisms by which microorganisms bind metal ions, in particular Fe, are considered to be an integral part of the series of events leading to the fossilization of cells (Ferris,

F. G., Fyfe, W. S., and Beveridge, T. J., 1988, Metallic ion binding in *Bacillus subtilis*: Implications for the fossilization of microorganisms: Geology, v. 16, p. 149-152. 1988; Mann, H., and Fyfe, W. S., 1989, Metal uptake and Fe-, Ti-oxide biomineralization by acidophilic microorganisms in mine-waste environments, Elliot Lake, Canada: Canadian Journal of Earth Sciences, v. 26, p. 2731-2735.). In addition, the mineralization of organisms may reflect the availability of dissolved solutes in the water column (Konhauser, K.O., Fyfe, W.S., Ferris, F.G., and Beveridge, T.J., 1993, Metal sorption and mineral precipitation by bacteria in two Amazonian river systems: Rio Solimões and Rio Negro, Brazil, Geology, V. 21, p. 1103-1106). The mineralization of CRB cells and filaments appears to be identical to previously documented accounts of biomineralization. We did not observe replacement by other minerals such as clays or carbonates in other samples, as discussed by Tazaki, K., 1997, Biomineralization of layer silicates and hydrated Fe/Mn oxides in microbial mats: An electron microscopical study: Clays and Clay Minerals, v. 45, p. 203.