tions, the faulted fan was most likely built by many episodes of deposition over thousands to tens of thousands of years. For the three youngest fans, we have used the youngest average ^{10}Be:^{26}Al age as a limit for fan surface abandonment, cognizant of the limitations imposed by small sample sizes (which causes overestimate of the limit), boulder erosion (causes underestimate), and the assumption of no isotope inheritance at deposition (causes overestimate). A minimum duration of fan deposition can be estimated from the distribution of boulder ages; however, such an estimate is a function of analytic precision, the age distribution of boulders cropping out on the fan surface, and the number of boulders sampled and analyzed. If more samples are collected from any single fan surface, the apparent duration of deposition should generally increase as the likelihood of sampling boulders from the tails of the age distribution increases.

5. S. Beanland and M. Clark, *U.S. Geol. Surv. Bull. 1982* (1994).
6. Thermoluminesence (TL)–derived ages at Lone Pine were not useful for determining recurrence intervals because the TL system appeared not to have been reset to zero during erosion and transport of material from the scarp to the adjacent graben. No organic material suitable for radiocarbon dating has been found in four trenches dug so far across the LPF and adjacent graben; it is likely that the highly oxidizing environment destroyed most organic material.
7. Quartz was separated [C. P. Kohl and K. Nishiizumi, *Geochim. Cosmochim. Acta* **56**, 3583 (1992)] and ^{10}Be and ^{26}Al were isolated by cation exchange after HF dissolution at the University of Vermont. We measured isotopic ratios at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory. Measured ratios, corrected for blanks and carrier, ranged from 15×10^{-15} to 1989×10^{-15} and 52×10^{-15} to 3509×10^{-15} for $^{10}Be/Be$ and $^{26}Al/Al$, respectively. Isotope production, as a function of altitude and latitude, is scaled for nucleon abundance (*15*); for samples reported here, production is 2.4 to 4.2 times that at sea level.
8. P. Larsen *et al.*, *Geol. Soc. Am. Abstr. Programs* **27**, 63 (1995).
9. K. Nishiizumi *et al.*, *J. Geophys. Res.* **94**, 17907 (1989).
10. New radiocarbon data from the Sierra Nevada suggest that ^{10}Be and ^{26}Al calibration sites (*9*) are about 20% older than originally believed (13,000 to 14,000 calendar years ago versus 11,000 calendar years ago); thus, ^{10}Be and ^{26}Al production rates at sea level and high latitude may be up to 20% lower than the previously accepted values of 6.03 and 36.8 atoms g^{-1} $year^{-1}$, respectively (D. H. Clark *et al.*, *Geol. Soc. Am. Abstr. Programs* **26**, A447 (1994); D. Clark *et al.*, *Quat. Res.*, in press). This finding is supported by ^{10}Be and ^{26}Al data from the Laurentide terminal moraine in New Jersey (*8*). If production rates are revised downward, our model ages will increase by similar percentages. In addition to uncertainties in average production rates, changes in geomagnetic field strength have probably caused instantaneous production rates at Sierra Nevada altitudes and latitudes to vary over the duration of boulder exposure on the fan surfaces. Because production-rate calibration was performed on late Pleistocene surfaces (*9*), exposure ages could be overestimated by 10 to 15% for samples from the Qg1 surface, first exposed during a period of relatively high isotope production, and underestimated by a similar magnitude for samples from the Qg4 surface, which were exposed in part during a period of high field strength and consequently low production rate. We interpret isotope abundances as ages using 20% uncertainty in production rates propagating the following uncertainties: 10%, age of calibration surface (*9*); 6%, abundance variance in calibration samples (*9*); 10%, variation in magnetic field strength over time (*9*); 10%, altitude and latitude correction (*15*); 5%, muon contribution to production; and 5%, geomagnetic latitude of the sample over time. All in situ cosmogenic ages cited in this report are in ^{10}Be, ^{26}Al, and ^{36}Cl years which reflect an uncertain and nonlinear stretching of the calendar time scale.
11. K. Nishiizumi *et al.*, *Earth Surf. Processes Landforms* **18**, 407 (1993).
12. P. R. Bierman *et al.*, *Geology* **19**, 641 (1991).
13. F. M. Phillips *et al.*, *Science* **248**, 1529 (1990).
14. F. Phillips *et al.*, *Geol. Soc. Am. Abstr. Programs* **25**, A373 (1993); T. Swanson *et al.*, *ibid.*, p. A461.
15. D. Lal, *Earth Planet. Sci. Lett.* **104**, 424 (1991).
16. We thank J. Turner for assistance in sample preparation, C. Massey, D. Clark, K. Whipple, and K. Campbell for field assistance, and the staff at the Center for Accelerator Mass Spectrometry (Lawrence Livermore National Laboratory), in particular, J. Southon, for assistance in making the isotopic measurements. TL measurements made by G. Berger. Reviews by M. Clark and M. Pavich greatly strengthened this manuscript. Supported by NSF grant EAR 9004252 and 9396261, U.S. Geological Survey grant 14-08-001-G1783, and the University of Vermont (P.R.B.), and by NSF grant EAR 9004252 and the Geology program at the National Aeronautics and Space Administration (A.R.G.).

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Lithoautotrophic Microbial Ecosystems in Deep Basalt Aquifers

Todd O. Stevens* and James P. McKinley

Bacterial communities were detected in deep crystalline rock aquifers within the Columbia River Basalt Group (CRB). CRB ground waters contained up to 60 μM dissolved H_2 and autotrophic microorganisms outnumbered heterotrophs. Stable carbon isotope measurements implied that autotrophic methanogenesis dominated this ecosystem and was coupled to the depletion of dissolved inorganic carbon. In laboratory experiments, H_2, a potential energy source for bacteria, was produced by reactions between crushed basalt and anaerobic water. Microcosms containing only crushed basalt and ground water supported microbial growth. These results suggest that the CRB contains a lithoautotrophic microbial ecosystem that is independent of photosynthetic primary production.

The existence of microorganisms in the deep terrestrial subsurface has been noted for decades (*1*); viable microorganisms are present at depths as great as several thousand meters below the surface, in broadly variable physical and chemical settings (*2*). Nutrient flux at such depths is usually very low because of limitations of sediment chemistry and hydrology. The few measurements of in situ metabolic rates from these systems are the lowest recorded, which indicates that although microorganisms are active at such depths, they function in Earth's most oligotrophic environments (*3*). Most reported subsurface communities are ultimately, though indirectly, dependent on photosynthesis for energy; they either use remnant organic carbon deposited with sediments or use dissolved oxygen as a metabolic terminal electron acceptor. As nutrients are exhausted from sediments, the enclosed microbial population should become extinct. Here, we report evidence for an active, anaerobic subsurface lithoautotrophic microbial ecosystem (SLiME) within the CRB that appears to derive energy from geochemically produced hydrogen. SLiMEs should persist independently of photosynthetic products.

The CRB is a series of Miocene tholeiitic continental flood basalts that formed 6 to 17 million years ago and cover >163,000 km^2 (*4*). In our study area (Fig. 1), the CRB is 3 to 5 km thick. With increasing depth, the age of the water in confined aquifers between basalt flows increases (ages may exceed 35,000 years), as does the lateral distance to recharge. Shallow ground waters are low-sulfate, low-chloride bicarbonate solutions of moderate pH (generally 7.5 to 8.5), with calcium as the dominant cation. At depth, sodium and chloride predominate, and pH varies from 8 to 10.5 (*4*, *5*). Sulfate concentrations are below 0.5 mM even at depth, except in geographically restricted zones where sulfate concentrations may exceed 2.0 mM. The igneous rocks in the study area contained little organic carbon, yet we found relatively high populations of anaerobic microorganisms within aquifers hundreds of meters below any sedimentary interbeds (*6*).

To identify the electron acceptors and electron donors to which CRB communities are adapted (*7*), we investigated the metabolic capabilities of bacteria from eight aquifers. We measured the population sizes of bacteria capable of dissimilatory Fe(III) reduction (DIRB), sulfate reduction (SRB), methanogenesis (MB), fermentation (FB), or acetogenesis (AB). We also compared numbers of organisms that could grow on simple organic compounds (heterotrophs) with numbers of organisms that could grow with H_2 as the sole electron donor (autotrophs). The aquifers were sampled (*8*) through a series of preexisting wells (Fig. 1). The results of geochemical measurements (Table 1) were consistent with microbiological measurements (Table 2). DIRB were present only at low numbers, FB were com-

Pacific Northwest Laboratory, Post Office Box 999, Richland, WA 99352, USA. E-mail: to_stevens@pnl.gov or jp_mckinley@pnl.gov

*To whom correspondence should be addressed.

mon, and AB and MB were ubiquitous, although MB numbers were low in the high-sulfate ground waters. In contrast, SRB numbers were high primarily in the high-sulfate waters. In nearly every sample, autotrophic microorganisms outnumbered heterotrophic microorganisms by several orders of magnitude. Although H_2 is a common bacterial electron donor, this finding contrasts with observations of a variety of subsurface sediments and surface soils (9).

High concentrations of dissolved methane have been observed locally in the CRB (Fig. 2A) and natural gas was commercially exploited early in this century, but the origin of the gas is uncertain; stable isotope data suggest that it is largely of biogenic origin (*10*). Hydrogen concentrations are also relatively high (Fig. 2B), mostly three or more orders of magnitude above the range of 0.05 to ~10 nM that would be expected from microbial fermentation of organic matter (*11*, *12*). Thus, dissolved H_2 is widely present in the CRB in nonlimiting concentrations sufficient to promote microbial metabolism (*12*).

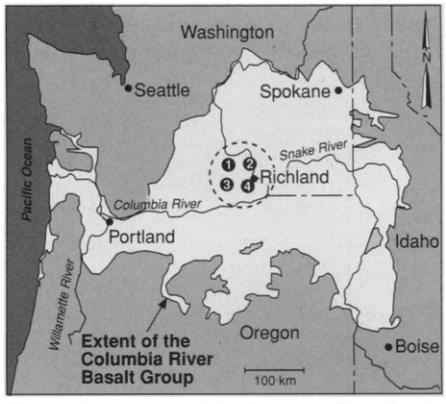

Fig. 1. Areal extent of the Columbia River Basalt Group and locations of the sampling wells used in this study. An extensive array of monitoring wells (within the dotted circle) was used for Figs. 2 and 3. Wells used for Tables 1 and 2 were located at numbered points (1, DB; 2, DC; 3, Pr; and 4, Jw).

Stable carbon isotope ratios of dissolved inorganic carbon (DIC) and methane suggest that the organisms in CRB ground waters are indeed active in situ. DIC is increasingly enriched in ^{13}C (*13*) at depths greater than ~200 m, consistent with the preferential removal of ^{12}C by methanogenic microorganisms (Fig. 3A). A smaller group of ground waters is depleted in ^{13}C and contains >0.5 mM dissolved sulfate; this observation suggests that sulfate-reducing bacteria oxidize biologically fixed carbon, which is relatively rich in ^{12}C. Thus, the electron acceptors available in the aquifer control the composition of microbial communities in the CRB.

During the production of methane, carbon is isotopically fractionated between methane and DIC. If the metabolism of H_2 and CO_2, as opposed to the metabolism of organic matter (acetate fermentation), were the primary process controlling methane formation, the effects of this process should be reflected both in concentrations of DIC and in the stable isotope compositions of that carbon. A characteristic depletion of $\Delta\delta^{13}C_{CO_2\text{-}CH_4} \approx -60$ parts per mil may be expected when bacteria form methane by reduction of CO_2 ($H_2 + CO_2 = CH_4$), as opposed to lesser fractionation ($\Delta\delta^{13}C_{CO_2\text{-}CH_4} \approx -20$ to -40 parts per mil) when bacteria grow on acetate ($C_2H_4O_2 = CH_4 + CO_2$), through which electron flow would occur during organic matter fermentation. To evaluate the hypothesis that CO_2 reduction was the dominant methanogenic pathway in CRB ground waters, we applied a fractionation model to existing data (*14*). Concentrations of DIC were assumed to decrease as a result of methanogenesis (Fig. 3B), and the concomitant expected change in $\delta^{13}C_{DIC}$ was calculated for comparison with measured $\delta^{13}C_{DIC}$. As shown in Fig. 3C, $\delta^{13}C_{DIC}$ values predicted by this method agreed with measured values. Calculations of expected $\delta^{13}C_{DIC}$ resulting from the acetate fermentation pathway ($C_2H_4O_2 = CH_4 + CO_2$) could not be adequately constrained.

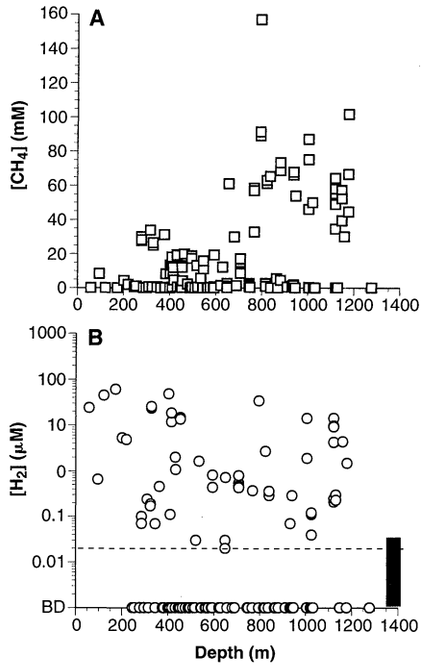

Fig. 2. (**A**) Dissolved methane and (**B**) hydrogen in CRB ground waters. Most data are from (*36*), with concentrations calculated after (*10*). These data were derived from an extensive system of monitoring wells located within the circled area in Fig. 1. In (B), the data are on a logarithmic scale (BD, below detection limits), the dashed line indicates the detection limit, and the vertical bar at the right represents the range of hydrogen concentrations expected during microbial oxidation of organic matter in sediments (*11*). Five different sampling methods were used to obtain these samples, including 26% of the total by artesian flow. There were no statistical differences between values obtained with different sampling methods, which suggests that sampling artifacts were minimal (*12*).

Table 1. Geochemical properties of ground-water samples from eight CRB aquifers. Well location codes are from Fig. 1; well numbers differentiate multiple wells at each locale. NA, data not available; <, below detection limit. Concentrations are millimolar, except as noted.

Analyte	Well number and solute values								Detection limit
	DC-06	DB-11	Jw-1	Jw-2	Jw-3	Pr-3	Pr-4	Pr-5	
Na	11.600	1.368	1.429	1.136	1.586	2.486	1.980	3.654	0.009
K	0.223	0.273	0.252	0.221	0.297	0.279	0.257	0.356	0.011
Ca	0.059	0.375	0.464	0.813	0.556	0.515	0.770	0.101	0.003
Mg	0.014	0.300	0.825	0.895	0.791	0.338	0.518	0.053	0.003
H_4SiO_4	1.789	0.958	1.022	0.956	1.086	0.799	0.769	1.020	0.003
F	1.905	0.040	0.025	0.029	0.024	0.040	0.027	0.076	0.001
Cl	4.568	0.120	0.167	0.174	0.179	0.281	0.261	0.240	0.002
NO_3	<	<	0.007	0.004	<	<	<	<	0.0002
SO_4	1.484	<	0.215	0.618	0.376	0.004	0.021	<	0.0005
Dissolved O_2	<	<	<	0.019	<	<	<	<	0.006
Sulfide (μM)	31.600	0.200	36.200	<	93.300	13.900	9.800	4.200	0.100
CH_4 (μM)	2	209	25	NA	16	481	135	185	1
pH	9.92	7.94	7.86	7.50	7.74	8.06	8.04	8.67	

Because DIC decreases with depth and acetate fermentation produces CO_2, an arbitrary sink for excess CO_2 was required in the calculations. The dependence of $\delta^{13}C_{DIC}$ on both addition and removal of DIC prevented a quantitative evaluation and argued against this mechanism as a control of $\delta^{13}C_{DIC}$. The success of the fractionation model lends further support to the hypothesis that abiotically produced H_2 supports microbial communities in the CRB. In the CRB, anaerobic H_2 oxidizers may be primary producers of organic carbon rather than facilitators of the catabolic terminal electron-accepting process, as they are in standard models of sediment metabolism (*15*).

We hypothesized that H_2O reduction to produce H_2, driven by iron in ferromagnesian silicates within the CRB, could serve as the abiotic energy source in this microbial ecosystem (*16*). We based this hypothesis on occurrences of free H_2 associated with ultramafic bodies in Earth's crust. H_2 has been observed at high concentrations (>10 volume % of exsolved gas) in nonvolcanic environments associated with serpentinized mafic and ultramafic rocks (*17, 18*). These occurrences apparently result from the weathering of Fe(II)-bearing silicates (such as olivine and pyroxene) at high rock:water ratios, where the dissolution of ferrous silicates and the precipitation of magnetite (Fe_3O_4) and other secondary phases drive the formation of H_2 (*19*). Weathering reactions may also be responsible for the alkalinity of ground waters observed in association with ultramafic bodies (*20*), and they can proceed and generate H_2 at ambient temperatures (*18, 21*). These proposed mechanisms are extrapolations from field observations and thermodynamic calculations; the precise reactions that are responsible for H_2 generation have not been demonstrated in controlled laboratory experiments. However, it is reasonable to expect that similar reactions would take place in CRB aquifers (*22*).

Ferrous silicates are not as abundant volumetrically in the CRB as in ultramafic rocks, but H_2 is also present at concentrations far below saturation in this system, which implies a much lower H_2 production rate. However, the abundance of ferrosilicate minerals does not necessarily limit the volume of product that can accumulate, because redox conditions during water-rock interaction are a function of the presence of ferrosilicates and dissolved oxygen, and not of mineral composition or volumetric abundance (*19*). The concentration of H_2 in CRB aquifers may be limited by the reacting surface area per unit volume of ground water and by the abundance of bacteria that make use of H_2.

In an attempt to produce direct evidence for H_2 production from basalt-water reactions, we conducted experiments with a variety of crushed rock samples. Steel is a common component of drill cuttings because of the abrasion of sampling and processing tools, and Fe(0) in steel may interact with water to produce H_2 (*23*). To rule out this source of hydrogen, we prepared steel-free crushed basalt samples from a well-characterized CRB outcrop (*24*) with the use of only stone and ceramic tools. Other rock samples were prepared in the same way. When basalt was added to pH-buffered water in sealed tubes under strictly anaerobic sterile conditions in the dark,

Table 2. Numbers of microorganisms in the eight CRB ground-water samples (om, organisms that grew on organic substrates; H_2, organisms that grew on $H_2 + CO_2$ only). The data are logarithms of numbers of organisms per milliliter belonging to various functional groups by enrichment series. The maximum detection limit was 10^4 organisms (+, growth in enrichments containing filters through which 500 ml of water was passed; –, no growth).

Sample	DIRB		SRB		MB		FB	AB
	om	H_2	om	H_2	om	H_2	om	H_2
DB-11	–	–	1	1	2	2	1	4
DC-06	+	+	2	4	+	1	4	4
Jw-1	+	–	3	4	1	4	1	4
Jw-2	+	–	2	+	1	2	1	4
Jw-3	+	–	1	1	1	1	2	4
Pr-3	+	–	4	3	2	4	3	3
Pr-4	–	–	+	1	2	4	2	4
Pr-5	–	–	1	1	1	4	3	4

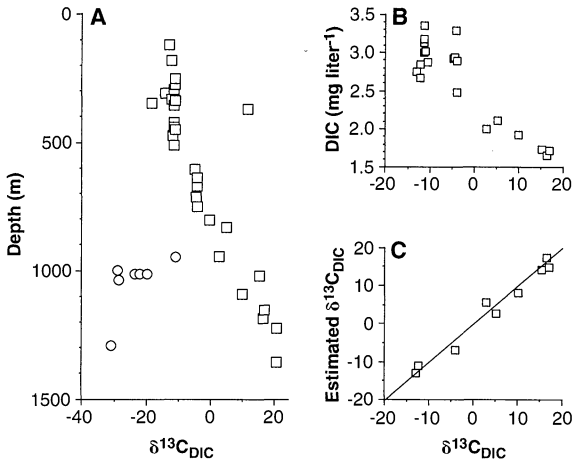

Fig. 3. Stable carbon isotopic signatures of confined aquifer samples. (**A**) Compositional variation with depth (○, high-sulfate samples). $\delta^{13}C_{DIC}$ generally increases with depth, which suggests carbon fixation by methanogenic bacteria, but in high-sulfate ground waters, $\delta^{13}C_{DIC}$ decreases, which suggests oxidation of biologically fixed carbon by sulfate-reducing bacteria. Compositions from less than 500 m below the land surface are relatively uniform and apparently have been little affected by methanogenesis. (**B**) $\delta^{13}C_{DIC}$ values increase with decreasing DIC. (**C**) $\delta^{13}C_{DIC}$ values of ground waters predicted by a fractionation model (*14, 37*) are nearly identical to measured values ($y = 1.009x + 0.005$, $r^2 = 0.928$). Results are plotted for samples that originated at depths below ~300 m and for which DIC concentration and $\delta^{13}C_{DIC}$ were known.

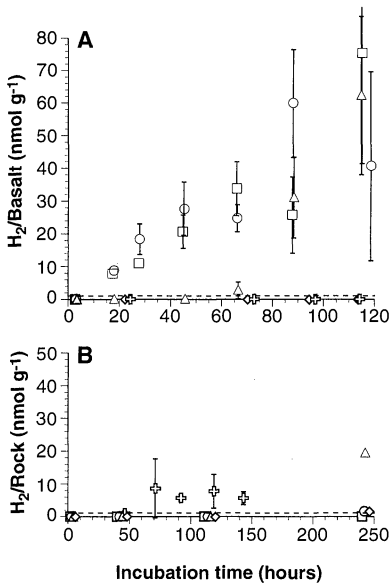

Fig. 4. Hydrogen gas production by in vitro basalt-water reactions. (**A**) Hydrogen produced by three basalt samples at 22°C [□, Umtanum Ridge basalt (*24*); ○, unweathered Cerro Negro basalt (from near Mount Taylor, NM); △, Snake River Plain basalt (from near Idaho Falls, ID); ✣, Umtanum basalt control with no buffer; ◇, buffer control with no basalt]. (**B**) Hydrogen production by other rocks [□, sandstone; ○, highly weathered Cerro Negro basalt dike 1; △, highly weathered Cerro Negro basalt dike 2; ◇, highly weathered Cerro Negro basalt dike 3; ✣, a granitic sample (from the Jurassic Ponder Pluton, British Columbia, Canada) containing ~10 volume % hornblende and 11 volume % biotite]. The data are the mean ± SD of three or more replicates; dashed lines indicate detection limits.

rapid H_2 evolution occurred (Fig. 4A) (*25*). The reactions occurred at room temperature and pressure and were completely inhibited by air (*26*). Similar results were obtained with basalt samples collected and processed on different days and with basalt samples collected from different regions. No H_2 was produced from basalt alone or buffer solution alone in control experiments, which indicated that the results were not attributable to the liberation of gases trapped in vesicles. Moreover, no H_2 was produced in assays with low-reactivity materials such as sandstone (Fig. 4B), and only trace amounts of H_2 were produced after extended incubation with highly weathered basalt samples, which should be depleted of reactants. A marked lag time consistently preceded H_2 evolution in a low-glass sample of Snake River Plain basalt, which then proceeded at the fastest measured rate; this finding suggests that more than one mineral phase is involved in this phenomenon. That is, some initial reactions (for instance, lowering the solution redox potential) by one phase may be required to establish favorable conditions for H_2 evolution by another phase. A granitic sample produced small amounts of H_2 after a 50-hour lag time, which indicates that at least some other rocks that contain ferrous silicate can also react to produce H_2.

If H_2-producing reactions occur in situ in the CRB and in similar crystalline rock formations, this phenomenon represents a widespread potential energy source for microbial metabolism in the subsurface. We speculate that unreacted mineral phases are exposed to ground water by ongoing microfracturing, the slow advancement of weathering fronts, and microbiologically enhanced weathering. These processes should result in regional production of potential microbial electron donors.

To determine whether basalt-water reactions alone could support microbial metabolism, we prepared a series of microcosms that contained only sterile crushed basalt and CRB ground water with its entrained microbial flora (*27*). A representative high-methane, low-sulfate ground water and a low-methane, high-sulfate ground water were used. As shown in Table 3, microorganisms proliferated in these microcosms. Most functional groups initially increased in number by several orders of magnitude, but with increasing time, autotrophic organisms maintained high numbers while heterotrophic microorganisms declined. This was true even in the high-sulfate microcosms where electron acceptors were not limiting. No microorganisms could be detected in microcosms that contained basalt and sterile buffer; in microcosms not containing basalt, no viable microorganisms could be detected after 24 weeks. Although microcosms might be subject to a "bottle effect," such an artifact would affect heterotrophs and autotrophs equally. The data suggest that growth and survival was promoted by geochemical H_2 production from basalt and ground water.

The prevalence of AB in these cultures seems unusual. Nearly all bacteria known to be capable of growing as AB can also grow as FB, although this was not indicated in our data. The role of $H_2 + CO_2$ acetogenesis in nature is not clear (*28*); it yields even less energy than methanogenesis, and MB should dominate AB in competition for H_2. In most sediments, AB activity accounts for only a few percent of in situ electron flow (*28*). We hypothesize that AB may successfully compete in the CRB aquifers because of the high H_2 concentrations. In this system, AB, as well as SRB and MB growing on $H_2 + CO_2$, could function as primary producers of organic matter.

The above results lead us to propose that a SLiME is largely responsible for the dissolved methane within the CRB. Because energy sources and inorganic nutrients both can be supplied by geochemical means in situ, microorganisms in SLiMEs can potentially persist in the deep terrestrial subsurface indefinitely. Several investigators (*29*, *30*) have speculated about possible lithoautotrophy in the subsurface. We have shown evidence for such a system and for a purely geochemical energy source. Furthermore, high temperatures or upwelling of geothermal fluids (*29*, *30*) are not required. The occurrence of SLiMEs may be widespread where appropriate mineralogic and physical conditions exist. For instance, extensive microbial populations have been reported in granitic aquifers in Sweden and Canada (*31*); these findings might be explained by reactions such as those in Fig. 4B. Anomalous concentrations of H_2 and CH_4 that include a bacteriogenic component have been reported in granitic terrains throughout the Canadian and Fennoscandian shields (*32*).

Autotrophic metabolism coupled to mineral weathering is an unusual geomicrobiological relation that has broad implications for microbial ecology. No other ecosystem is currently known to exist independently of past or present photosynthesis (*33*). SLiMEs conceivably provide a model for the existence of contemporary life on Mars because basalt, liquid water, and bicarbonate are believed to be present in the martian subsurface (*29*, *34*). SLiMEs may also provide a model for how surface organisms could have lived on Earth before the evolution of photosynthesis and the development of an oxidizing atmosphere about 2.8 billion years ago (*35*).

Table 3. Numbers of microorganisms in basalt–ground-water microcosms as a function of incubation time. The data are logarithms of numbers of organisms per milliliter belonging to various functional groups by enrichment series. The maximum detection limit was 10^4 organisms (–, no growth). DB-11 microcosms contained low-sulfate ground water; DC-06 microcosms contained high-sulfate ground water.

Microcosm (well)	Time (days)	DIRB		SRB		MB		FB	AB
		om	H_2	om	H_2	om	H_2	om	H_2
DB-11	0	–	–	1	1	2	2	1	4
	14	1	1	–	–	4	4	4	4
	49	1	1	–	–	4	4	4	4
	77	1	1	–	–	2	4	3	4
	238	–	2	–	–	–	4	–	4
	350	–	2	–	–	–	4	–	4
DC-06	0	–	–	2	4	–	1	4	4
	14	1	1	4	4	–	–	4	4
	49	–	–	3	4	–	–	3	4
	77	1	1	2	4	–	–	2	4
	238	1	–	2	4	–	–	2	4
	350	–	–	2	4	–	–	2	4

REFERENCES AND NOTES

1. A. M. Buswell and T. E. Larson, *J. Am. Water Works Assoc.* **29**, 1978 (1937); C. E. Zobell, *Am. Assoc. Pet. Geol. Bull.* **31**, 1709 (1947); S. I. Kuznetsov, M. V. Ivanov, N. N. Lyalikova, *Introduction to Geological Microbiology*, P. T. Broneer, Transl. (McGraw-Hill, New York, 1963).
2. For reviews of much recent work, see *Geomicrobiol. J.* **7** (no. 1, 1988) and references therein; *J. Microbiol. Methods* **21** (March 1995) and references therein; *Proceedings of the First International Symposium on Microbiology of the Deep Subsurface*, Orlando, FL, 15 to 19 January 1990 (WSRC Information Services, Aiken, SC, 1990); K. Pedersen, *Earth Sci. Rev.* **34**, 243 (1993); E. L. Madsen and W. C. Ghiorse, in *Aquatic Microbiology*, T. E. Ford, Ed. (Blackwell, Boston, 1993), pp. 167–213.
3. F. H. Chapelle and D. R. Lovley, *Appl. Environ. Microbiol.* **56**, 1865 (1990); T. J. Phelps, E. M. Murphy, S. M. Pfiffner, D. C. White, *Microbiol. Ecol.* **28**, 335 (1994).
4. "Consultation Draft, Site Characterization Plan, Reference Repository Location, Hanford Site, Washington" (DOE/RW-0164, U.S. Department of Energy, Washington, DC, 1988).
5. P. P. Hearn Jr., W. C. Steinkampf, L. D. White, J. R. Evans, *Proc. U.S. Geol. Surv. Workshop on Environmental Geochemistry* (USGS Circ. 1033, 1990), pp. 63-68.
6. T. O. Stevens, J. P. McKinley, J. K. Fredrickson, *Microbiol. Ecol.* **25**, 35 (1993).
7. Functional groups of bacteria were enumerated as described in (*6*), except that decimal dilution series

were used to estimate numbers of organisms and additional enrichment series were prepared for each electron acceptor. In the additional series, hydrogen was provided as the sole electron donor by pressurizing the headspace with an oxygen-free 50:50 mixture of H_2 and CO_2 at 2 bar.

8. Wells, either artesian or pumped, were purged for at least three well volumes and until field measurements (pH, Eh, conductivity) stabilized. Sample containers were thoroughly flushed with formation water and then aseptically filled and tightly sealed with butyl stoppers. This procedure was not as reliable as aseptically obtained core samples from the aquifers would have been; however, the agreement of microbiological results with regional ground-water signatures indicated that the results were representative of in situ aquifer communities.
9. T. O. Stevens *et al.*, unpublished data.
10. V. G. Johnson, D. L. Graham, S. P. Reidel, *Am. Assoc. Pet. Geol. Bull.* **77**, 1192 (1993); J. P. McKinley, T. O. Stevens, J. K. Fredrickson, in preparation.
11. D. R. Lovley and S. Goodwin, *Geochim. Cosmochim. Acta* **52**, 2993 (1988). Five sedimentary interbeds containing 0.01 to 0.6% organic carbon are present in our study area and may contribute to the ecology of the upper parts of the CRB. We incubated samples of each interbed with CRB ground water and its entrained microorganisms in anaerobic microcosms for up to 2 years to determine whether organic matter in interbeds could promote microbial fermentation and subsequent production of biomass and hydrogen. Sediments from two interbeds could promote the survival and growth of typical anaerobic communities, although, as expected, elevated H_2 concentrations did not result. These interbeds are minor components of the CRB and are unlikely to be responsible for the amount of biomass we observed. In any case, in our study area, high concentrations of both H_2 and bacteria (*6, 36*) were observed hundreds of meters below the deepest interbed. Thus, fermentation of organic matter in interbeds cannot explain the regional observations discussed above.
12. Artificially high hydrogen measurements can result from the use of stainless steel submersible pumps during sampling [D. R. Lovley, F. H. Chapelle, J. C. Woodward, *Environ. Sci. Technol.* **28**, 1205 (1994)]; because 36% of our samples were obtained with these pumps, individual values from the data set should be accepted with caution. The data did not show any correlation of H_2 concentration with depth sampled, production rate, or sampling method, and hence H_2 was likely not a sampling artifact.
13. Isotopic signatures are expressed in standard notation. The departure of the sampled $^{13}C/^{12}C$ ratio (R) from that of the standard material (Pee Dee belemnite), in parts per mil, is calculated according to

$$\delta^{13}C_{DIC} = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 1000$$

14. Predicted $\delta^{13}C_{DIC}$ values were calculated with a Rayleigh fractionation model that conformed to the zero input–one output model of Wigley *et al.* (*37*),

$$(\delta^{13}C_{DIC} + 1000) = (\delta^{13}C_{DIC_0} + 1000)\,(mC/mC_0)^{\alpha-1}$$

where mC/mC_0 is the ratio of measured DIC to initial DIC and α is a fractionation factor. Carbon removed from ground water as methane was assumed not to further interact with DIC. Initial DIC (C_0) and $\delta^{13}C_{DIC_0}$ were fixed at values predominant within the aquifers less than 500 m below the land surface (2.75 mmol liter^{-1} and −13 parts per mil, respectively) and α was fixed at 1.060. In nature, fractionation of methane relative to DIC varies from −40 to −90 parts per mil (*38*), corresponding to α values of 1.040 to 1.090. The fraction of DIC remaining for each calculation was determined as mC/mC_0 and the resultant calculated $\delta^{13}C_{DIC}$ was compared with the directly measured value (Fig. 3).
15. A. J. B. Zehnder, Ed., *Biology of Anaerobic Microorganisms* (Wiley, New York, 1988).
16. Migration of H_2 from deeper in Earth's crust is possible; however, H_2 concentrations did not appear to vary with depth (Fig. 2), as would be expected if diffusion from below were the major source.
17. E. E. Angino, R. M. Coveney, E. D. Goebel, E. J. Zeller, G. Dreschhoff, *Oil Gas J.* **82**, 142 (1984); T. A. Abrajano *et al.*, *Chem. Geol.* **71**, 211 (1988).
18. C. Neal and G. Stanger, *Earth Planet. Sci. Lett.* **66**, 315 (1983).
19. P. Ramdohr, *Neues Jahrb. Mineral. Abh.* **107**, 241 (1967).
20. H. W. Nesbitt and O. P. Bricker, *Geochim. Cosmochim. Acta* **42**, 403 (1978); I. Barnes and J. R. O'Niel, *Geol. Soc. Am. Bull.* **80**, 1947 (1969).
21. I. Barnes, J. R. O'Niel, J. J. Trescases, *Geochim. Cosmochim. Acta* **42**, 144 (1978); P. Fritz, I. D. Clark, J.-C. Fontes, M. J. Whiticar, E. Faber, in *Water-Rock Interaction*, Y. K. Kharakha and A. S. Maest, Eds. (Balkema, Rotterdam), pp. 793–796; J. A. Apps, in *Lawrence Berkeley Laboratory, Earth Sciences Division Annual Report 1984* (U.S. Department of Energy, Berkeley, CA, 1985), pp. 13–17.
22. A well-studied example of abundant H_2 that may have resulted from mafic rock-water interaction occurs in Kansas. H_2 abundances in excess of 30 volume % were found to have originated through shallow low-temperature water-rock interaction; although kimberlites are known to occur approximately 50 km from wells containing H_2, no proximate ultramafic rocks have been identified (*18*) [E. D. Goebel and G. A. M. Dreschhoff, *Oil Gas J.* **82**, 215 (1984); R. M. Coveney Jr., E. D. Goebel, E. J. Zeller, G. A. M. Dreschhoff, E. E. Angino, *Am. Assoc. Pet. Geol. Bull.* **71**, 39 (1977)]. Serpentinization reactions involving mafic igneous rocks known to exist in the Precambrian basement near the wells have been suggested as the source of H_2. These authors speculated from this association that H_2-containing gases may be more common than previously recognized, because of generation by weathering of mafic minerals in the subsurface; our data tend to confirm this inference. H_2 evolution from ground hedenbergite, olivine, and dunite in a poorly controlled experiment was reported by V. I. Molchanov, *Dokl. Akad. Nauk SSSR* **182**, 192 (1968).
23. In a previous report, accidental H_2 generation was suspected to have resulted from a crushed CRB slurry, but possible steel contamination was also implicated [B. N. Bjornstad *et al.*, *Ground Water Monitor. Remed.* **14**, 140 (fall 1994)].
24. The outcrop of Umtanum Ridge basalt was a site designated BWIP-EC RUC.1 4-29-80 [C. C. Allen, R. G. Johnston, M. B. Strope, "Characterization of Reference Umtanum and Cohassett Basalt" (RHO-SD-BWI-DP-053, U.S. Department of Energy, Richland, WA, 1985)]. The mineralogy of this outcrop was (by modal %) plagioclase, 36.2; pyroxene, 18.1; mesostasis, 38.2; titaniferous magnetite, 4.6; apatite, 0.98; and alteration products, 0.31. Basalt was excavated from the outcrop with large basalt cobbles as tools. Samples were reduced to 2-cm size by hurling cobbles together; these fragments were then crushed with ceramic-faced mechanical sample crushers and sieved to <0.25 mm by means of polyethylene sieves with polyester mesh. The samples were examined by light microscopy and by x-ray diffraction analysis of magnetically separated fines. No steel contamination was detected.
25. Reaction conditions for data shown were: temperature, 22°C; headspace, O_2-free N_2 at 1 bar; basalt sample, 5 g, particle size <0.25 mm; solution, 5 ml oxygen-free 1 mM sodium phosphate buffer, pH 6.0. Basalt aliquots were placed in 20-ml glass pressure tubes filled with N_2 gas and sealed with gas-impermeable butyl-rubber stoppers and aluminum crimp seals [W. E. Balch and R. S. Wolfe, *Appl. Environ. Microbiol.* **32**, 781 (1976)]. To avoid addition of exogenous H_2, we did not use the usual heated copper catalyst. Tubes were autoclaved for 30 min at 121°C, allowed to cool to room temperature overnight, and reautoclaved to obtain sterile samples. The autoclaving process was not required for H_2 production (T. O. Stevens and J. P. McKinley, data not shown) but was used to reduce variability resulting from the growth of random incidental microorganisms and to rule out biological contributions to hydrogen production. Small amounts of H_2 produced during autoclaving were removed by flushing sterile tubes with N_2 gas introduced through a 0.2-μm syringe filter and a hypodermic needle while venting through another needle. Sterile anaerobic buffer was prepared by boiling under N_2 gas and cooling to room temperature before dispensing into N_2-flushed pressure tubes, sealing, and autoclaving. Experiments were initiated by transferring an aliquot of buffer into a tube of basalt with a sterile, N_2-flushed, hypodermic needle and syringe. Hydrogen was measured by gas chromatography of a headspace sample, with either a Hewlett-Packard 5890-II gas chromatograph with a thermal conductivity detector (for micromolar concentrations) or a Trace Analytical RGA3 reduction-gas analyzer with a mercury-reduction detector (for nanomolar concentrations). Routine controls included basalt samples to which no buffer was added and buffer aliquots with no basalt sample.
26. T. O. Stevens and J. P. McKinley, data not shown.
27. Microcosms were sterile 160-ml serum bottles with butyl-rubber stoppers and aluminum seals. Each contained 50 g of autoclaved basalt chips (size <4 mm) and was filled with ground water at the wellhead, leaving no headspace, and sealed with sterile butyl stoppers. Microcosms were incubated in the dark at room temperature for up to 1 year and were sampled periodically by sacrificing individual microcosms. Assays were performed as described in (*7*).
28. J. Dolfing, in (*15*), pp. 417–468; H. L. Drake, Ed., *Acetogenesis* (Chapman and Hall, New York, 1994).
29. P. J. Boston, M. V. Ivanov, C. P. McKay, *Icarus* **95**, 300 (1992) and references therein.
30. T. Gold, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6045 (1992).
31. K. Pedersen and S. Ekendahl, *Microbiol. Ecol.* **20**, 37 (1992); *ibid.* **23**, 1 (1992); *J. Gen. Microbiol.* **138**, 369 (1992); D. A. Brown, D. C. Kamineni, J. A. Sawicki, T. J. Beveridge, *Appl. Environ. Microbiol.* **60**, 3182 (1994).
32. B. Sherwood-Lollar *et al.*, *Geochim. Cosmochim. Acta* **57**, 5087 (1993); *ibid.*, p. 5073.
33. The well-known deep-sea hydrothermal vent communities depend on photosynthetically produced dissolved O_2 as their terminal electron acceptor. It is possible, however, that a system similar to what we describe here exists in the subsurface basalt surrounding the vents.
34. S. W. Squyres and J. F. Kasting, *Science* **265**, 744 (1994); C. P. McKay, E. I. Friedman, R. A. Wharton, W. L. Davies, *Adv. Space Res.* **12**, 231 (1992); "An Exobiological Strategy for Mars Exploration," *NASA SP-530* (1995).
35. H. D. Holland, *The Chemical Evolution of the Atmosphere and Oceans* (Princeton Univ. Press, Princeton, NJ, 1984); J. W. Schopf, Ed., *Earth's Earliest Biosphere* (Princeton Univ. Press, Princeton, NJ, 1983); J. W. Schopf and C. Klein, Eds., *The Proterozoic Biosphere* (Cambridge Univ. Press, Cambridge, MA, 1992); S. Bengston, Ed., *Early Life on Earth* (Columbia Univ. Press, New York, 1994).
36. T. O. Early, G. D. Spice, M. D. Mitchell, "A Hydrochemical Data Base for the Hanford Site, Washington" (RHO-SD-BWI-DP-061, U.S. Department of Energy, Richland, WA, 1986).
37. T. M. L. Wigley, L. N. Plummer, F. J. Pearson Jr., *Geochim. Cosmochim. Acta* **42**, 1117 (1978); *ibid.* **43**, 1395 (1979).
38. M. J. Whiticar, E. Faber, M. Schoell, *Geochim. Cosmochim. Acta* **50**, 693 (1986); L. M. Games and J. M. Hayes, in *Environmental Biogeochemistry*, J. O. Nriagu, Ed. (Ann Arbor Science, Ann Arbor, MI, 1976), vol. 1, pp. 51–73.
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