peak seen along the [10] direction of the reciprocal space whose spatial frequency corresponds to one-third of the [10] spot frequency. In direct space we observed that, perpendicular to the [10] direction, one molecular row in every three is slightly higher than the two others by about 0.2 Å (see Fig. 1D). Moreover, in the case of Fig. 1D, we observed a slight modulation of the spacing between rows perpendicular to the buckling direction. The amplitude of this modulation was estimated to be about 4% of the average row spacing. This modulation appears in the 2-D Fourier transform of Fig. 1D through the relative amplitude of the peaks along the [10] direction. Computer calculations performed on numerically defined lattices allowed us to model this 2-D Fourier transform. We were only able to simulate the relative amplitudes of the peaks along the [10] direction by taking into account at the same time a modulation of row height and a slight modulation of row spacing. Whereas the height modulation was observed systematically over the whole sample, the very subtle changes of row spacings were found to vary from one location to another on the sample. The molecular organization described above, in the case of the remaining bilayer, is found for any layer of the layered domains.

In order to rule out any artifact, we show in Fig. 4A a grain boundary observed within the same sample (*19, 20*). On each side of the boundary we observed two domains with exactly the same structure (a modulated, centered rectangular lattice) rotated by an angle of 20° (see Fig. 4B). Furthermore, such a structure was never observed on substrates (annealed or not silanated silicon wafers). We conclude that this modulation is an intrinsic crystallographic property of the annealed LB films.

From these observations, we conclude that the disordered hexagonal structure observed for unannealed samples is a metastable state. The previously measured extent of the positional order in unannealed systems is obviously related to the presence of dislocations that perturb this positional order. Those defects probably originate from constraints during the transfer of the monolayer on water onto the solid substrate. These conclusions are strongly favored by our observations that the molecular structure is not affected by the number of incorporated cations. Upon annealing, the LB films reorganize into a more ordered state of higher density, in which the area per AA molecule is almost identical to the one measured in the AA monolayer on water. The dewetting [the mechanism of which has yet to be elucidated (*21, 22*)] and the three-dimensional reconstruction of the LB film observed at macroscopic scales originate from the relaxation of internal stresses attributable to surface energy; this relaxation is responsible for the observed modulation of the molecular structure and could be due to a buckling phenomenon constrained by the LB film lattice.

REFERENCES AND NOTES

1. K B. Blodgett and I. Langmuir, *Phys Rev* **55**, 19 (1937)
2. M. Sugi, *J. Mol. Electron.* **1**, 3 (1985)
3. G. Khanarian, *Thin Solid Films* **152**, 265 (1987)
4. W. M. Reichert, C. J Bruckner, J Joseph, *ibid*, p 345.
5. L. Bourdieu, P. Silberzan, D Chatenay, *Phys. Rev. Lett.* **67**, 2029 (1991).
6. J A N. Zasadzinski *et al.*, *Biophys. J* **59**, 755 (1991).
7. H. G. Hansma *et al.*, *Langmuir* **7**, 1051 (1991)
8. E Meyer *et al.*, *Nature* **349**, 398 (1991)
9. J P K Peltonen *et al*, *J Am Chem. Soc.* **114**, 7637 (1992).
10. M. Seul, P. M. Eisenberger, H. M McConnell, *Proc. Natl. Acad. Sci. U S A.* **80**, 5795 (1983).
11. M. L. Schlossmann *et al.*, *Phys. Rev Lett* **66**, 1599 (1991).
12. K. Kjaer, E. E. Mitchell, I. R. Peterson, *Thin Solid Films* **205**, 124 (1991).
13. S Garoff, H W Deckmann, J H Dansmuir, M. S. Alvarez, *J Phys. (Paris)* **47**, 701 (1958).
14. R. Steitz, E E Mitchell, I. R Peterson, *Thin Solid Films* **205**, 124 (1991).
15. A Fisher and E. Sackmann, *J Colloid Interface Sci.* **112**, 1 (1986)
16. P Silberzan, L Léger, D. Ausserré, J J. Benattar, *Langmuir* **7**, 1674 (1991).
17. J Daillant, J. J Benattar, L. Léger, *Phys. Rev. A* **41**, 1963 (1990).
18. C Vogel *et al.*, *J. Chim. Phys Phys. Chim. Biol.* **77** (no. 10), 947 (1980).
19. P Tippmann-Krayer, H Mohwald, Yu M. L'vov, *Langmuir* **7**, 2298 (1991)
20. J Garnaes, D. K. Schwartz, R Viswanathan, J A. N. Zasadzinski, *Nature* **357**, 54 (1992)
21. F Brochard Wyart and J Daillant, *Can. J. Phys.* **68**, 1084 (1990)
22. G. Reiter, *Phys. Rev Lett* **68**, 75 (1992).

23. This work was supported by CNRS (GDR 912 "Films moléculaires flexibles" and GDR 936 "Forces de surface"). We have benefited from insightful comments from J. Prost, J. Meunier, V. Croquette, M. Goldman, and B Factor

25 September 1992, accepted 30 November 1992

Mediation of Sulfur Speciation by a Black Sea Facultative Anaerobe

K. A. Perry, J. E. Kostka, G. W. Luther III, K. H. Nealson

Shewanella putrefaciens, a respiratory facultative anaerobe isolated from the Black Sea, can reduce thiosulfate, sulfite, and elemental sulfur to sulfide readily and quantitatively. This widespread and anaerobically versatile microorganism, which is incapable of reducing sulfate, uses oxidized sulfur intermediates as electron acceptors during the respiratory oxidation of organic matter. Because of its widespread distribution and abundance, it may play a significant role in sulfur and trace metal cycling in the Black Sea and in other marine and freshwater anaerobic environments.

Recent studies have shown sulfur intermediates to be integral to electron flow between oxic and anoxic environments (*1*), although most microbiological studies involving the marine sulfur cycle have concentrated on sulfate (SO_4^{2-}) reduction or the oxidation of sulfur intermediates. *Shewanella putrefaciens* is a facultative anaerobe found in oxic, suboxic, and anoxic water columns and sediments. It is capable of utilizing a number of electron acceptors including O_2, Mn(IV), Fe(III), NO_3^-, NO_2^-, and a variety of sulfur compounds (*2, 3*). However, it cannot reduce SO_4^{2-}. To examine the contribution of this bacterium to the sulfur cycle, we grew a strain of *S. putrefaciens* isolated from the Black Sea (MR-4) anaerobically in pure culture with one of the following electron acceptors: thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), or zerovalent sulfur (S^0) (*4*). We measured the inorganic sulfur species SO_3^{2-}, $S_2O_3^{2-}$, particulate S^0, and sulfide (HS^-) (*5, 6*) and cell density (*7*) for up to 140 hours after inoculation.

In all cultures, the electron acceptor was the only detectable sulfur species at time zero, and the decrease in its concentration with time corresponded with an increase in both HS^- and cell density (Figs. 1 to 3). The reduction of $S_2O_3^{2-}$ appeared to involve initial disproportionation of the molecule, as evinced by the appearance of SO_3^{2-} in early stages of the culture. Concentrations of SO_3^{2-} reached a maximum at 10 to 12 hours after inoculation in all replicates (Fig. 1), indicating that the bacteria first disproportionated the $S_2O_3^{2-}$ either to S^0 and SO_3^{2-} (Table 1, reaction 1), or to HS^- and SO_3^{2-} (Table 1, reaction 2), or with an electron donor such as lactate (Table 1, reaction 3). The SO_3^{2-} and S^0 (if formed) were then reduced to HS^-. All of these reactions are thermodynamically unfavorable and must be coupled to exergonic reactions such as carbon oxidation. Our data do not indicate conclusively

K A Perry, J E Kostka, G W. Luther III, College of Marine Studies, University of Delaware, Lewes, DE 19958.

K H Nealson, Center for Great Lakes Studies, University of Wisconsin, Milwaukee, WI 53204.

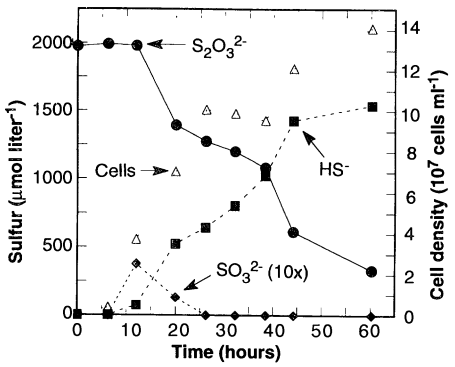

Fig. 1. Growth of *S. putrefaciens* with $S_2O_3^{2-}$ (1 mmol liter^{-1}) (S, 2 mmol liter^{-1}) as the sole electron acceptor. Thiosulfate is reported as micromoles of sulfur per liter. The mass balance of all sulfur species measured was 100% of the sulfur added at all times (within an analytical error of 10%).

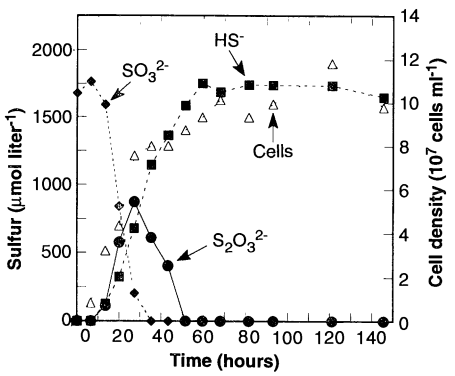

Fig. 2. Growth of *S. putrefaciens* with SO_3^{2-} (1.7 mmol liter^{-1}) as the sole electron acceptor. Thiosulfate is plotted as micromoles of sulfur per liter. The mass balance of all sulfur species measured was 100% of the sulfur added at all times (within an analytical error of 10%).

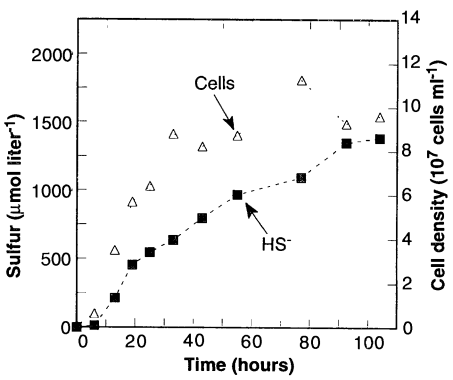

Fig. 3. Growth of *S. putrefaciens* with S^0 (approximately 10 mmol liter^{-1}) as the sole electron acceptor.

which mechanism the bacteria use for transformation of $S_2O_3^{2-}$. Particulate S^0 was not detected at any time in the culture and, because the mass balance of the sulfur species was always maintained, it is unlikely that any colloidal S^0 formed. Therefore, if $S_2O_3^{2-}$ was disproportionated according to reaction 1 (Table 1), the S^0 must have been reduced rapidly to HS^-. As the culture aged, SO_3^{2-} was not detectable because it was either immediately reduced to HS^- or reacted with the HS^- to reform $S_2O_3^{2-}$ by the reverse of reaction 2 (Table 1). This reaction occurs readily and is used to prepare $S_2O_3^{2-}$ (*8*). The disproportionation of $S_2O_3^{2-}$ shown here is different from that described by Bak and Cypionka (9), as the SO_4^{2-}-reducing bacteria that they studied disproportionated $S_2O_3^{2-}$ either to S^0 and SO_4^{2-} or to HS^- and SO_4^{2-} (Table 1, reaction 4). The latter process cannot be occurring in the *S. putrefaciens* cultures because the mass balance of all the sulfur species was constant; thus, no SO_4^{2-} was formed. The culture stopped logarithmic phase growth after approximately three-quarters of the $S_2O_3^{2-}$ had been reduced because HS^- reached inhibitory levels (*10*).

Thiosulfate appeared early on in cultures given SO_3^{2-} as the sole electron acceptor and achieved a maximum concentration at approximately 25 hours in all replicates (Fig. 2). Fitz and Cypionka (*11*) suggested that $S_2O_3^{2-}$ and trithionate ($S_3O_6^{2-}$) are intermediates in a three-step reduction of SO_3^{2-} by SO_4^{2-}-reducing bacteria. We never observed $S_3O_6^{2-}$ in any culture (it would give a polarographic wave), and, because S^0 was also not seen, the reverse of reaction 2 (Table 1), as discussed above, is the most likely explanation for the appearance of $S_2O_3^{2-}$. The SO_3^{2-} was thus rapidly depleted both by reduction to HS^- and by reaction with HS^- to form $S_2O_3^{2-}$, which was subsequently reduced to HS^-. In both the SO_3^{2-} and $S_2O_3^{2-}$ cultures, sulfur cycled between SO_3^{2-} and $S_2O_3^{2-}$ before it was completely reduced to HS^-.

Shewanella putrefaciens was also grown on freshly precipitated S^0, which consisted of a mixture of S_n (n = 1 to 10) (*12*). Sulfide was detected within 10 min after inoculation and increased concomitantly with cell density (Fig. 3). These results indicate that the bacteria were reducing S^0 to HS^-. Because of the difficulties incurred in measurement of both colloidal and particulate S^0, a mass balance could not be determined for these cultures; however, neither SO_3^{2-} nor $S_2O_3^{2-}$ was ever detected. As SO_4^{2-} apparently never formed when *S. putrefaciens* was grown on either $S_2O_3^{2-}$ or SO_3^{2-}, it is unlikely that it would form when the bacteria were given S^0.

This bacterium joins several other groups of bacteria that can utilize S^0 as an electron acceptor. However, it is the only non-SO_4^{2-}-reducing bacterium that has been demonstrated to grow with SO_3^{2-} or $S_2O_3^{2-}$ as the terminal electron acceptor (*13, 14*). The ability of this non-SO_4^{2-}-reducing bacterium to reduce S^0 and $S_2O_3^{2-}$ may be environmentally significant. As HS^- diffuses upward from anoxic environments, it is oxidized chemically by contact with dissolved species (oxygen and nitrate) and solid phases (iron and manganese oxides), as well as biologically by chemosynthetic bacteria; S^0 and $S_2O_3^{2-}$ are the predominant oxidation products (*1, 15–23*). Although usually found in low concentrations, $S_2O_3^{2-}$ appears to be important in coupling the oxidative and reductive pathways of the sulfur cycle, and thus it is an important species controlling electron flow in sediments (*1, 21–24*). The respiratory versatility of *S. putrefaciens* may account for the absence of sulfur species of intermediate oxidation state such as $S_2O_3^{2-}$, SO_3^{2-}, $S_4O_6^{2-}$, and S^0 in anoxic marine environments such as the Black Sea, where anaerobic oxidation of HS^- has been observed (*25*). Many bacteria are capable of removing these sulfur species: SO_4^{2-} reducers reduce and disproportionate them (*9, 26*), and, in the presence of appropriate electron acceptors, lithotrophic sulfur bacteria can oxidize them (*27, 28*). However, Nealson *et al.* (*29*) could not find any evidence of SO_4^{2-} reduction activity in

Table 1. Reactions involving sulfur species of intermediate oxidation state. Reactions 1 to 4 represent disproportionation of thiosulfate. Reactions 5 to 7 summarize the use of $S_2O_3^{2-}$, SO_3^{2-}, and S^0 as terminal electron acceptors by *S. putrefaciens*, with lactate as an electron donor. The standard free energy change of the reaction at 1 atm, 25°C, and pH 7 ($\Delta G^{0\prime}$) is reported as kilojoules per mole of sulfur.

	Reaction	$\Delta G^{0\prime}$ (kJ mol^{-1})
1	$\frac{1}{2}S_2O_3^{2-} \leftrightarrow \frac{1}{2}S^0 + \frac{1}{2}SO_3^{2-}$	+16.10
2	$\frac{1}{2}S_2O_3^{2-} + \frac{1}{2}H_2O \leftrightarrow \frac{1}{3}HS^- + \frac{2}{3}SO_3^{2-} + \frac{2}{3}H^+$	+30.98
3	$\frac{1}{2}S_2O_3^{2-} + \frac{1}{4}CH_3CHOHCOO^- + \frac{1}{2}H_2O \leftrightarrow \frac{1}{2}HS^- + \frac{1}{2}SO_3^{2-} + \frac{1}{4}CH_3COO^- + \frac{1}{4}HCO_3^- + \frac{3}{4}H^+$	+1.01
4	$\frac{1}{2}S_2O_3^{2-} + \frac{1}{2}H_2O \leftrightarrow \frac{1}{2}HS^- + \frac{1}{2}SO_4^{2-} + \frac{1}{2}H^+$	−8.26
5	$\frac{1}{2}S_2O_3^{2-} + CH_3CHOHCOO^- + \frac{1}{2}H_2O \rightarrow HS^- + CH_3COO^- + HCO_3^- + H^+$	−88.88
6	$SO_3^{2-} + \frac{3}{2}CH_3CHOHCOO^- \rightarrow HS^- + \frac{3}{2}CH_3COO^- + \frac{3}{2}HCO_3^- + \frac{1}{2}H^+$	−179.79
7	$S^0 + \frac{1}{2}CH_3CHOHCOO^- + H_2O \rightarrow HS^- + \frac{1}{2}CH_3COO^- + \frac{1}{2}HCO_3^- + \frac{3}{2}H^+$	−30.17

enrichment cultures down to a depth of 500 m in Black Sea cultures, approximately 400 m below the suboxic-anoxic interface. Although Jørgensen *et al.* (*27*) found that $S_2O_3^{2-}$-oxidizing bacteria composed 0.01 to 0.6% of the total bacterial numbers near the interface (10^1 to 10^3 cells per milliliter), *Shewanella* species made up 20 to 50% of the total bacterial counts in the suboxic zone (10^5 cells per milliliter) (*29*). Therefore, the lack of HS^- oxidation products in the Black Sea and in other environments where SO_4^{2-} reducers are absent may be due largely to the presence of *S. putrefaciens*. This microorganism may also be important where SO_4^{2-} reducers are present because of its ability to utilize a wider range of carbon substrates (*2*, *3*).

The manipulation of sulfur species by *S. putrefaciens* can be summarized by reactions 5 to 7 in Table 1. The respiratory versatility of *S. putrefaciens* may give it a distinct metabolic advantage in suboxic and anoxic environments such as reducing sediments or stratified basins like the Black Sea, where the concentrations of various electron acceptors change with time and depth. This microorganism may play an important role in both trace metal and sulfur cycling between oxic and anoxic environments. Although *S. putrefaciens* readily reduces solid-phase iron and manganese, it also utilizes the more soluble oxidation products of HS^- (for example, $S_2O_3^{2-}$), released as iron and manganese oxides settle and react with HS^-.

Jørgensen and co-workers (*1*, *21–23*) have demonstrated that the $S_2O_3^{2-}$ shunt plays an important role in coupling the oxidative and reductive pathways of the sulfur cycle in both marine and freshwater environments. The discovery of an abundant group of organisms that can utilize $S_2O_3^{2-}$ and other partially oxidized sulfur species as electron acceptors provides additional biological mechanisms for establishing electron flow through sulfur compounds. These findings indicate that there is a direct, enzymatic connection between marine sulfur and carbon cycles, which is separate from SO_4^{2-} reduction.

REFERENCES AND NOTES

1. B. B. Jørgensen, *Science* **249**, 152 (1990).
2. C. R Myers and K. H. Nealson, *ibid.* **240**, 1319 (1988).
3. _____, *Geochim. Cosmochim. Acta* **52**, 2727 (1988).
4. Cultures were grown under Ar in autoclaved, filtered (0.2 μm) seawater enriched with yeast extract (0.2 g liter^{-1}) and bacto-peptone (0.1 g liter^{-1}) with filter-sterilized 30 mM acetate, 30 mM lactate, and 2 mM $NaHCO_3$ added. The medium was deaerated and inoculated with approximately 10^5 cells per milliliter, and then the deaerated, filter-sterilized electron acceptor was added. The pH was 7 4 to 7.6 Culture growth was monitored by acridine orange direct counting of cells Control cultures in which either the electron acceptor or the bacteria were excluded showed no significant growth.
5. G. W. Luther III, A. E. Giblin, R. Varsolona, *Limnol. Oceanogr.* **30**, 727 (1985). We measured SO_3^{2-}, $S_2O_3^{2-}$, and HS^- by square wave voltammetry of filtered media, HS^- was also determined by colorimetry. Particulate S^0 was measured by methanol extraction of 0.2-μm Nuclepore membranes and high-performance liquid chromatography of the extract with ultraviolet detection. We did not measure SO_4^{2-} because the media already contained approximately 28 mM SO_4^{2-}, and small concentration changes would not be detectable. As the mass balance of the sulfur species (ΣS = $[S_2O_3^{2-}]$ + $[SO_3^{2-}]$ + $[S^0]$ + $[HS^-]$) always added up to 100% of the sulfur added (within an analytical error of 10%) without SO_4^{2-}, this was deemed acceptable. All sampling was done in an Ar-filled glove bag.
6. J. D. Cline, *Limnol. Oceanogr.* **14**, 454 (1969).
7. J. E. Hobbie, R. J. Daley, S. Jasper, *Appl. Environ. Microbiol.* **33**, 1225 (1977).
8. N. N. Greenwood and A. Earnshaw, *Chemistry of the Elements* (Pergamon, Toronto, 1984).
9. F. Bak and H. Cypionka, *Nature* **326**, 891 (1987).
10. K. H. Nealson, unpublished material.
11. R. M. Fitz and H. Cypionka, *Arch. Microbiol.* **154**, 400 (1990).
12. R. Steudel, T. Gobel, G. Holdt, *Z. Naturforsch. Teil B* **43**, 203 (1988). We prepared elemental sulfur under Ar by precipitating sterile concentrated $S_2O_3^{2-}$ with H_2SO_4 followed by centrifugation, resuspension in fresh deaerated sterile deionized water, and salting out with deaerated sterile saturated NaCl The precipitate was then recentrifuged, resuspended, and reprecipitated six additional times to assure that only zerovalent sulfur was present.
13. G. Fauque, J. Legall, L. L. Barton, in *Variations in Autotrophic Life*, J. Shively and L. L. Barton, Eds. (Academic Press, London, 1991), pp. 271–337.
14. J. Legall and G. Fauque, in *Biology of Anaerobic Microorganisms*, A. J. B. Zehnder, Ed. (Wiley, New York, 1988), pp. 587–639.
15. J. H. Tuttle and H. W Jannasch, *Limnol. Oceanogr.* **17**, 532 (1972).
16. _____, *Mar. Biol.* **20**, 64 (1973).
17. K. Y. Chen and J. C. Morris, *Environ. Sci. Technol.* **6**, 529 (1972).
18. A. J. Pyzik and S. E. Sommer, *Geochim. Cosmochim. Acta* **45**, 687 (1981).
19. D. J. Burdige and K. H. Nealson, *Geomicrobiol. J.* **4**, 361 (1986).
20. R. C. Aller and P. D. Rude, *Geochim. Cosmochim. Acta* **52**, 751 (1988).
21. B. B. Jørgensen, *Limnol. Oceanogr.* **35**, 1329 (1990).
22. H. Fossing and B. B. Jørgensen, *Geochim. Cosmochim. Acta* **54**, 2731 (1990).
23. B. B. Jørgensen and F. Bak, *Appl. Environ. Microbiol.* **57**, 847 (1991).
24. R. W. Howarth, *Biogeochemistry* **1**, 5 (1984).
25. G. W. Luther III, T. M. Church, D. Powell, *Deep-Sea Res.* **38** (Suppl. 2), S1121 (1991).
26. F. Widdel, in *Biology of Anaerobic Microorganisms*, A. J. B. Zehnder, Ed. (Wiley, New York, 1988), pp. 469–585.
27. B. B. Jørgensen, H. Fossing, C. O. Wirsen, H. W. Jannasch, *Deep-Sea Res.* **38** (Suppl. 2), S1083 (1991).
28. H. W. Jannasch, C. O. Wirsen, S. J. Molyneaux, *ibid.*, p. S1105.
29. K. H. Nealson, C. R. Myers, B. B. Wimpee, *ibid.*, p. S907.

30. This research was supported by the National Science Foundation (G.W.L. and K.H.N.) and by the Petroleum Research Foundation administered by the American Chemical Society (G.W.L.).

2 September 1992· accepted 3 December 1992

Multiple Evolutionary Origins of Magnetotaxis in Bacteria

Edward F. DeLong,* Richard B. Frankel, Dennis A. Bazylinski*†

Magnetosomes are intracellular, iron-rich, membrane-enclosed magnetic particles that allow magnetotactic bacteria to orient in the earth's geomagnetic field as they swim. The magnetosomes of most magnetotactic bacteria contain iron oxide particles, but some magnetotactic species contain iron sulfide particles instead. Phylogenetic analyses of small subunit ribosomal RNA sequences showed that all known magnetotactic bacteria of the iron oxide type are associated with the α subgroup of the Proteobacteria in the domain Bacteria. In contrast, uncultured magnetotactic bacteria of the iron sulfide type are specifically related to the dissimilatory sulfate-reducing bacteria within the δ subdivision of the Proteobacteria. These findings indicate a polyphyletic origin for magnetotactic bacteria and suggest that magnetotaxis based on iron oxides and iron sulfides evolved independently.

Magnetotaxis in bacteria (*1*) is the result of a permanent magnetic dipole moment that causes a cell to be oriented in the earth's geomagnetic field as it swims (*2*). The magnetic dipole moment is caused by the presence of magnetosomes (*3*), intracellular, iron-rich, membrane-enclosed magnetic particles. In most magnetotactic bacteria, the particles consist of the ferrimagnetic iron oxide magnetite (Fe_3O_4) (*4–8*). However, some magnetotactic bacteria collected from sulfidic, brackish-to-marine aquatic habitats have recently been found to contain iron sulfide particles (*9*, *10*), including ferrimagnetic greigite (Fe_3S_4) (*9*, *11*, *12*) and nonmagnetic pyrite (FeS_2) (*9*, *11*). Although magnetotactic bacteria of both the iron oxide and iron sulfide types are frequently found in the same habitats, they grow in discrete locations, as indicated

E. F. DeLong, Department of Biology, University of California, Santa Barbara, CA 93106.
R. B. Frankel, Department of Physics, California Polytechnic State University, San Luis Obispo, CA 93407.
D. A. Bazylinski, Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

*To whom correspondence should be addressed.
†Present address: Department of Chemistry and Chemical Engineering, Stevens Institute of Technology, Castle Point on the Hudson, Hoboken, NJ 07030.