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Nitrate Dissimilation Under Microaerophilic Conditions by a Magnetic Spirillum

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During microaerophilic growth of magnetic spirillum MS-1 on tartrate and nitrate, a maximal cell density was obtained at an initial oxygen partial pressure of 17 Pa. A transient accumulation of nitrous oxide and a 1:2 (mol/mol) stoichiometry between tartrate oxidation and nitrate reduction were observed, indicating that the organism carried out a respiratory type of metabolism.

The isolation of magnetic spirillum MS-1 and its culture in a chemically defined medium have been described recently (5). This organism has been shown by electron microscopy to contain electron-dense particles aligned along the major axis of the cell comprising a novel intracellular structure, the magnetosome (2). Frankel et al. have shown that the major constituent of the magnetosome is magnetite (6). Results reported here indicate that this microaerophile possesses a respiratory type of metabolism in which nitrate may serve as the electron acceptor under microaerophilic conditions.

The bacterium was grown on a chemically defined medium as previously described (5) with the following modifications: 250 μmol of L-(+)-sodium tartrate (Sigma Chemical Co.) per 50 ml of medium was the sole carbon source; succinate, acetate, and ammonium chloride were omitted. Oxygen utilization, nitrous oxide evolution, and total CO_2 produced by growing cells were monitored by injecting an aseptically obtained sample of the gas phase from a culture vial into a Packard Gas Chromatograph model 428 furnished with an electron capture detector, a Porapak Q column, and oxygen-free nitrogen as the carrier gas. The amount of each gas present was computed by comparison with previously established standards. Samples were aseptically withdrawn with a 100-μl A-2 Series Pressure-Lok syringe (Precision Sampling Co.). The total amount of CO_2 produced was determined at stationary phase after acidification of the culture; CO_2 initially present in the medium was determined by a similar procedure. Growth was estimated by measuring the increase in culture absorbance at 540 nm (1-cm light path) in a Gilford-Beckman DU spectrophotometer. The nitrate concentration in the medium was estimated by the method described by Wood et al. (11) with the following modifications: $CuSO_4 \cdot 5H_2O$ (2 g/100 ml of water) was made anaerobic by sparging the solution with oxygen-free nitrogen for 30 min. Cadmium filings were conditioned as described in the original method. Clean cadmium filings were poured into the cupric sulfate solution and allowed to stand under continuous bubbling and flushing until the solution turned colorless. The copperized cadmium was then transferred into an anaerobic chamber (1) and poured to form a column (8 by 120 mm). Samples were eluted from the column with 0.002 M ethylenediaminetetraacetic acid at a flow rate of 10 ml/min. A 2-ml sample of the eluate was diluted by the addition of 2 ml of 7.3 mM sulfanilamide in 1.25 M HCl and 2 ml of a 4.3 mM solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride (Marshall's reagent). The color was measured at 543 nm in a Bausch & Lomb Spectronic 20 spectrophotometer. The assay was accurate over the range of 10 to 130 nmol of nitrate.

Nitrate disappearance was followed under conditions where tartrate was limiting (Fig. 1A). As the cell density increased, nitrate was consumed and nitrous oxide accumulated in the gas phase of the culture vial. Cell lysis became significant after about 50 h. The amount of O_2 consumed by the microorganism, 7 μmol, was quite small compared to the amount of nitrate consumed, 463 μmol. Furthermore, 936 μmol of CO_2 was recovered after 60 h, which is very close to the expected value of 1,000 μmol for complete utilization of the tartrate added. The overall equation for the oxidation of tartrate to CO_2 with the reduction of nitrate is (7, 10):

$$C_4H_4O_6^{2-} + 6H_2O \rightarrow 4HCO_3^- + 10e + 12H^+$$
$$2NO_3^- + 10e + 12H^+ \rightarrow N_2 + 6H_2O$$
$$\overline{C_4H_4O_6^{2-} + 2NO_3^- \rightarrow 4HCO_3^- + N_2}$$

The amount of reducing power generated by the oxidation of tartrate correlated well with the amount of anaerobically dissimilated nitrate. To demonstrate the transitory formation of nitrous

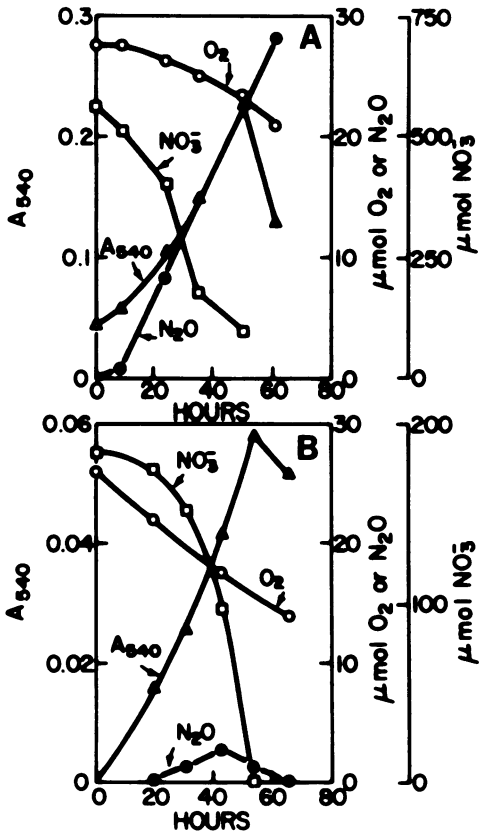

FIG. 1. *(A) Growth of magnetic spirillum MS-1 under tartrate-limiting conditions. Cells were grown in a 160-ml serum vial with 50 ml of chemically defined medium that contained 250 μmol of tartrate and 563 μmol of nitrate. (B) Growth of magnetic spirillum MS-1 under nitrate-limiting conditions. The medium was the same as for (A) except that 180 μmol of nitrate was added.*

oxide, a well-documented intermediate in dissimilatory nitrate reduction (9), the magnetic spirillum was grown under nitrate-limiting conditions (Fig. 1B). Nitrous oxide was only detected after the onset of nitrate utilization and rapidly increased to a maximum. Thereafter, the N_2O concentration decreased as the amount of nitrate in the medium became limiting. The evolution and eventual depletion of nitrous oxide indicated that the latter is an intermediate in nitrate respiration by this bacterium. Maximal cell density was obtained at an initial oxygen partial pressure of 17 Pa (absorbancy at 540 nm = 0.3), whereas at 35 Pa no growth occurred. In another experiment the following relationships were obtained: 2 Pa, absorbancy (at 540 nm) = 0.041; 4 Pa, absorbancy = 0.048; 8.5 Pa, absorbancy = 0.056; 34 Pa, absorbancy = 0.01. Consistent with previous results (5), we have been unable to by-pass the oxygen requirement for growth; the spirillum has an obligate requirement for oxygen but is an extreme microaerophile that is able to dissimilate nitrate. When nitrate was limiting (Fig. 1B), more oxygen was consumed than by a culture with an absorbance 3.7 times greater (Fig. 1A).

Of the many different kinds of magnetic bacteria that may be readily found in natural habitats (3, 4, 8), the magnetic spirillum is the only one so far to be reported in pure culture. One of the difficulties in isolating magnetic bacteria appears to involve their absolute requirement for oxygen despite their extreme sensitivity to more than trace amounts of it. Thus the role of magnetotaxis could be to direct the motile cell downward in aquatic environments to regions of lower oxygen tension, as suggested by Blakemore (3).

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LITERATURE CITED

1. **Aranki, A., and A. Freter.** 1972. Use of anaerobic glove boxes for the cultivation of strictly anaerobic bacteria. Am. J. Clin. Nutr. **25**:1329-1334.
2. **Balkwill, D. L., D. Maratea, and R. P. Blakemore.** 1980. Ultrastructure of a magnetotactic spirillum. J. Bacteriol. **141**:1399-1408.
3. **Blakemore, R. P.** 1975. Magnetotactic bacteria. Science **190**:377-379.
4. **Blakemore, R. P., R. B. Frankel, and R. S. Wolfe.** 1979. Ferromagnetism in freshwater bacteria, p. 400-407. *In* R. D. Phillips, M. F. Gillis, W. T. Kaune, and D. D. Mahlum (ed.), Biological effects of extremely low frequency electromagnetic fields. Proceedings of the Eighteenth Annual Hanford Life Sciences Symposium, 1978. Technical Information Center, U.S. Department of Energy, Washington, D.C.
5. **Blakemore, R. P., D. Maratea, and R. S. Wolfe.** 1979. Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium. J. Bacteriol. **140**:720-729.
6. **Frankel, R. B., R. P. Blakemore, and R. S. Wolfe.** 1978. Magnetite in freshwater magnetotactic bacteria. Science **203**:1355-1356.
7. **Hurlbert, R. E., and W. B. Jakoby.** 1965. Tartaric acid metabolism. I. Subunits of L(+) tartaric acid dehydrase. J. Biol. Chem. **240**:2772-2777.
8. **Moench, T. T., and W. A. Konetzka.** 1978. A novel method for the isolation and study of a magnetotactic bacterium. Arch. Microbiol. **119**:203-212.
9. **Payne, W. J.** 1973. Reduction of nitrogenous oxides by microorganisms. Bacteriol. Rev. **37**:409-452.
10. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100-180.
11. **Wood, E. D., F. A. J. Armstrong, and F. A. Richards.** 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. U.K. **47**:23-31.