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Aerobic respiration by two Sulfate reducing magnetotactic bacteria, strains RS-1 and FH-1

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Magnetotactic bacteria is the categorical name for a group of prokaryotes that biomineralize magnetosomes which are intracellular, membrane-bounded magnetic iron mineral crystals. The focus of this study is on two magnetite-producing, magnetotactic sulfate-reducing bacteria (SRB), *Desulfovibrio magneticus* strain RS-1 and strain FH-1 which also belongs in the genus *Desulfovibrio* in the *δ-Proteobacteria*. SRB utilize sulfate as a terminal electron acceptor under anaerobic conditions reducing sulfate to sulfide. A large number of organic compounds as well as some inorganic compounds have been shown to provide electrons for sulfate reduction. Traditionally, because no SRB have been shown to convincingly grow with O_2 as a terminal electron acceptor, they have been classified as obligate anaerobes.

In characterizing several magnetotactic SRB, we found that cells of *D. magneticus* and strain FH-1 utilized O_2 as an electron acceptor for growth. To prove this we grew cells of both strains in several different semi-solid growth media under air or N_2 gas. Cells of both strains grew as a microaerophilic band of cells at the oxic-anoxic interface (OAI) in media under air lacking sulfate (medium contained cysteine or cysteine with either Casamino Acids or Yeast Extract as a sulfur source). Sulfide (as FeS: high [Fe] was used as a trap for sulfide) was not produced in these tubes. Cells did not grow under anaerobic conditions (under N_2) in this medium unless sulfate was present. When sulfate was present in the growth medium, under air, initial growth of the strains was also as a microaerophilic band of cells at the OAI. However as time went on, the band of *D. magneticus* split into two. The band of FH-1 cells did not split into two bands and moved up the tube almost to the meniscus. The medium also turned dark indicating sulfide production. The results show that these magnetotactic SRB strains are capable of aerobic growth with O_2 as a terminal electron acceptor.

Aerobic Respiration by Two Sulfate Reducing Magnetotactic Bacteria, Strains RS-1 and FH-1

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Abstract

Magnetotactic bacteria is the categorical name for a group of prokaryotes that biomineralize magnetosomes which are intracellular, membrane-bounded magnetic iron mineral crystals. The focus of this study is on two magnetite-producing, magnetotactic sulfate-reducing bacteria (SRB), *Desulfovibrio magneticus* strain RS-1 and strain FH-1 which also belongs in the genus *Desulfovibrio* in the *δ-Proteobacteria*. SRB utilize sulfate as a terminal electron acceptor under anaerobic conditions reducing sulfate to sulfide. A large number of organic compounds as well as some inorganic compounds have been shown to provide electrons for sulfate reduction. Traditionally, because no SRB have been shown to convincingly grow with O_2 as a terminal electron acceptor, they have been classified as obligate anaerobes.

In characterizing several magnetotactic SRB, we found that cells of *D. magneticus* and strain FH-1 utilized O_2 as an electron acceptor for growth. To prove this we grew cells of both strains in several different semi-solid growth media under air or N_2 gas. Cells of both strains grew as a microaerophilic band of cells at the oxic-anoxic interface (OAI) in media under air lacking sulfate (medium contained cysteine or cysteine with either Casamino Acids or Yeast Extract as a sulfur source). Sulfide (as FeS: high [Fe] was used as a trap for sulfide) was not produced in these tubes. Cells did not grow under anaerobic conditions (under N_2) in this medium unless sulfate was present. When sulfate was present in the growth medium, under air, initial growth of the strains was also as a microaerophilic band of cells at the OAI. However as time went on, the band of *D. magneticus* split into two. The band of FH-1 cells did not split into two bands and moved up the tube almost to the meniscus. The medium also turned dark indicating sulfide production. The results show that these magnetotactic SRB strains are capable of aerobic growth with O_2 as a terminal electron acceptor.

Introduction

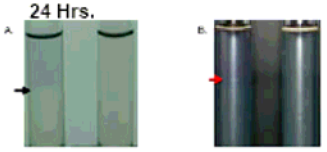
Magnetotactic bacteria (MTB) biomineralize intracellular, membrane-bounded, magnetic iron mineral crystals called magnetosomes which cause the cells to orient along the Earth's geomagnetic field. Magnetosomes contain either magnetite (Fe_3O_4) and/or greigite (Fe_3S_4). MTB are phylogenetically and metabolically diverse although most are affiliated with subgroups of the Proteobacteria particularly the alpha subgroup (1). MTB are known to be microaerophiles, anaerobes or both. Among the anaerobic MTB, *Desulfovibrio magneticus* strain RS-1 is a dissimilatory sulfate-reducing MTB that is phylogenetically associated with the *δ-Proteobacteria* (2). We recently isolated another sulfate-reducing MTB, called strain FH-1, that is related to *D. magneticus*, from a fish hatchery pond in Montana. Both strains produce Fe_3O_4 and are not known to produce Fe_3S_4 despite the fact they produce sulfide from sulfate.

The dissimilatory sulfate-reducing bacteria (SRB), those prokaryotes that reduce and grow anaerobically with sulfate as a terminal electron acceptor, are and have been generally regarded as obligate anaerobes (3). It has been shown that SRB have the ability to use many inorganic and organic compounds anaerobically as electron acceptors including nitrate, Fe^{3+}, fumarate, dimethylsulfoxide, and elemental sulfur (4). None have been definitively shown to grow with O_2 as a terminal electron acceptor although aerotaxis and tolerance to periods of O_2 exposure by SRB have been demonstrated (5). Some studies have also shown that some SRB contain respiratory chains that can reduce O_2 to water (6). However, because these strains did not grow on O_2, the reduction of O_2 was thought to have a protective function during brief exposures to O_2 (7). In this study, we show that cells of *D. magneticus* and strain FH-1 don't just tolerate O_2, but can utilize it as a terminal electron acceptor for growth. This is the first report of consistent aerobic growth of a SRB with O_2 as the terminal electron acceptor. This finding is critical in determining the important ecological roles MTB and SRB play in the environment.

Results

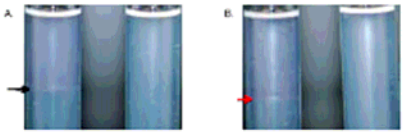
Cells of both strains grew as a microaerophilic band of cells at the oxic-anoxic interface (OAI) in media under air lacking sulfate (medium contained cysteine or cysteine with either Casamino Acids or Yeast Extract as a sulfur source). The main band of *D. magneticus* cells remained close to its original position as the OAI moved up the tube while a second, less pronounced band of cells moved up the tube towards the meniscus. In addition, the medium turned dark below the OAI indicating sulfide production and a band of sulfur formed near the meniscus of the tube. In contrast, the band of FH-1 cells did not split into two bands and moved up the tube almost to the meniscus. The medium also turned dark indicating sulfide production. Cells did not grow anaerobically in any medium unless sulfate was present.

24 Hrs.



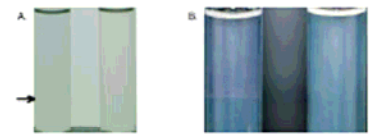
A) The arrow indicates the oxic-anoxic interface (OAI). In the aerobic tubes. Resazurin in the media indicates O2 in tubes by displaying a pink-purple color. Right anaerobic no sulfate-cys control.
B) Growth of *D. magneticus* as a microaerophilic band of cells at the OAI in medium without sulfate and cysteine-casamino acids as the sulfur source. Left aerobic; right anaerobic

72 Hrs.



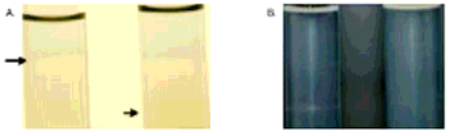
A) Growth of *D. magneticus* as a microaerophilic band of cells at the OAI (arrow) in medium without sulfate and cysteine-casamino acids as the sulfur source. Results were similar for FH-1. Left aerobic RS-1 casamino acids; right anaerobic RS-1 casamino acids
B) Growth of strain FH-1 as a microaerophilic band of cells at the OAI (arrow) in medium with sulfate and cysteine. Results were similar for *D. magneticus*. Left aerobic sulfate-cys; right anaerobic sulfate-cys.

120 Hrs.



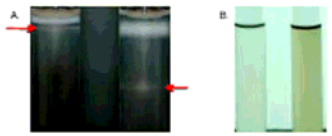
A) Left, uninoculated aerobic control tube showing the OAI at a lower position because of auto-oxidation of cysteine in the medium. Right, anaerobic cysteine control tube showing no indication of an OAI.
B) The OAI did not move downward in the aerobic, inoculated tubes that showed growth presumably because cells were taking up O_2. Cells of FH-1 growing as a band at the OAI at 120 h. Left aerobic Cys-yeast extract with no sulfate and right is anaerobic Cys-yeast extract with no sulfate.

168 Hrs.



A) The band of cells of strain FH-1 moved up the tube staying at the OAI. Thus, cells of FH-1 appear to prefer to use O_2 as an electron acceptor even in the presence of sulfate. The initial band of *D. magneticus* cells split into 2 bands, the main one remaining at its original position which is now in the anaerobic zone. The other less dense band moved up with the displacing OAI, seem to begin reducing sulfate as determined by the faint band in the anaerobic zone. Arrows indicate main band of cells. Left FH-1 aerobic sulfate; right RS-1 aerobic sulfate
B) Growth of *D. magneticus* in medium with cysteine alone as the sulfur source. Under aerobic conditions, cells grow as a microaerophilic band of cells. There is no growth in the absence of O_2. Similar results were obtained for strain FH-1. Left RS-1 aerobic cys no sulfate; right RS-1 anaerobic cys no sulfate.

240 Hrs.



A) The cells of FH-1 are at the OAI following the O_2 as the OAI moves up. RS-1 cells are clearly forming a band well below the OAI which is indicative of anaerobic growth by sulfate reduction. Left FH-1 sulfate aerobic; right RS-1 sulfate aerobic.
B) Left, uninoculated anaerobic control tube of medium with sulfate. Right, tubes shows sulfide production as black FeS by cells of FH-1 when grown anaerobically with sulfate as the electron acceptor. Similar results were obtained for *D. magneticus*.

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Methods

Growth Medium

Ingredients per liter (final concentrations in growth medium are in parentheses)

5 ml Wolfe's Modified Mineral Solution (all sulfate salts were replaced with chloride salts; the concentration of the metals were the same as in original Wolfe's Mineral Solution)

- 200 µl 0.2% Aqueous Resazurin solution
- 3 ml 0.5 M $KHPO_4$ buffer, pH 6.9 (1.5 mM)
- 0.082 g $MgCl_2 \cdot 7H_2O$ (0.4 mM)
- 0.17 g $NaNO_3$ (2 mM)
- 0.75 g sodium succinate-$6H_2O$ (3 mM)
- 0.142 g Na_2SO_4 (where present) (1 mM)
- 0.25 g of Casamino Acids (Difco) or Yeast Extract (Difco) (where present)

pH of the medium was adjusted to 6.87 with 0.1 N HCl and 2.0 g Bacto-Agar (Difco) was added after which the medium was heated to boiling and autoclaved. After autoclaving and cooling to ~45°C, the following was added to the growth medium.

- 4.0 ml freshly made, neutralized (with NaOH), filter-sterilized 0.3 M cysteine-HCl·H_2O solution (1.1 mM)
- 1.5 ml freshly made 0.8 M $NaHCO_3$ solution (the $NaHCO_3$ is autoclaved dry) (1.2 mM)
- 3.5 ml anaerobic 10 mM (low Fe) or 100 mM (high Fe) $FeCl_2 \cdot 4H_2O$ solution (dissolved in 0.02 N (low Fe) or 0.05 N (high Fe) HCl) (35 µM (low Fe) or 350 µM (high Fe))

10 ml of medium was dispensed into sterile 125 x 16 mm glass test tubes with butyl rubber septum stoppers (Bellco Glass Co.). Approximately 10⁶ cells were inoculated per tube while the medium was still liquid at ~43°. Tubes were then inverted several times to disperse the cells throughout the growth medium and then put on ice for about 3 min.

The headspace of aerobic cultures was left as air and for anaerobic cultures, the headspace was replaced with O_2-free N_2 by purging the headspace with N_2 (~150 ml per min) for 12 minutes per tube.

Conclusion

- Cells of strain FH-1 and *D. magneticus* grow as aerobes with O_2 as the terminal electron acceptor proving that some SRB are not strict obligate anaerobes.
- Cells of *D. magneticus* however seems to prefer using sulfate and grow as an anaerobe when given a choice between sulfate and O_2 while cells of strain FH-1 appear to prefer O_2 over sulfate.
- The fact that cells of FH-1 and *D. magneticus* respire and grow with O_2 supports the theory that even magnetotactic SRB like other MTB use magnetosomes to find the OAI more efficiently.

Future Outlook

- To do further studies measuring the rates of O_2 uptake by *D. magneticus* and FH-1.
- Determine the proteins and pathways involved in O_2 respiration in these magnetotactic SRB strains.
- Further investigate how aerobic respiration in these strains affects biomineralization of magnetosomes.

Acknowledgement
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