Diversity of Magneto-Aerotactic Behaviors and Oxygen Sensing Mechanisms in Cultured Magnetotactic Bacteria

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Supporting Material

MATERIALS AND METHODS

Isolation and growth of magnetotactic bacteria

Strains MSR-1, UT-4 and LM-1 were grown in the same freshwater medium in heterotrophic conditions. 5 ml of modified Wolfe's mineral elixir (1, 2), 0.2 ml 1% aqueous resazurin, 0.4 g NaCl, 0.3 g NH_4Cl, 0.1 g $MgSO_4$ • 7H2O, 0.05 g $CaCl_2$ • 2H2O, 1 g sodium succinate, 0.5 g sodium acetate and 0.2 g yeast extract (Difco Laboratories, Detroit, MI) were added to a liter of water, and the pH was adjusted to 7.0. A total of 1.6 g of Bacto agar (Difco Laboratories, Detroit, MI) was then added, after which the medium was autoclaved.

Strain RS-1 was grown in a freshwater medium in heterotrophic conditions. 5 ml of modified Wolfe's mineral elixir (1, 2) without sulfate, 0.2 ml 1% aqueous resazurin, 0.17 g $NaNO_3$, 0.082 $MgCl_2$ • $6H_2O$, 0.75 g sodium succinate and 0.25 g yeast extract. (Difco Laboratories, Detroit, MI) were added to a liter of water, and the pH was adjusted to 7.0. A total of 1.6 g of Bacto agar (Difco Laboratories, Detroit, MI) was then added, after which the medium was autoclaved.

Strains MV-1, PR-1, PR-2 and PR-3 were grown in the same saline medium in heterotrophic conditions. The formula of the artificial seawater was (per liter): 20 g NaCl, 6 g $MgCl_2$ • $6H_2O$, 3.24 g Na_2SO_4, 1 g $CaCl_2$ • $2H_2O$ and 0.5 g KCl. To a liter of this mixture, 5 ml of modified Wolfe's mineral elixir (1, 2), 0.2 ml 1% aqueous resazurin, 0.3 g NH_4Cl, 1 g sodium succinate, 2.4 g HEPES and 0.2 g yeast extract (Difco Laboratories, Detroit, MI) were added, and the pH was adjusted to 7.0. A total of 1.6 g of Bacto agar (Difco Laboratories, Detroit, MI) was then added, after which the medium was autoclaved.

Strains MC-1 and MMS-1 were grown in the same saline medium in autotrophic conditions. The formula of the artificial sea water was (per liter): 16.4 g NaCl, 3.5 g $MgCl_2 \cdot 6H_2O$, 2.8 g Na_2SO_4, 1 g $CaCl_2 \cdot 2H_2O$ and 0.5 g KCl. To a liter of this mixture, 5 ml of modified Wolfe's mineral elixir (1, 2), 0.2 ml 1% aqueous resazurin, 0.3 g NH_4Cl and 2.4 g HEPES were added, and the pH was adjusted to 7.0. A total of 1.6 g of Bacto agar (Difco Laboratories, Detroit, MI) was then added, after which the medium was autoclaved.

Strains SS-5 and SS-1 were grown in hypersaline media in autotrophic conditions using thiosulfate and sulfide, respectively as reduced sulfur compounds. The formula of the artificial seawater was (per liter): 37.8 g NaCl, 6 g $MgCl_2 \cdot 6H_2O$, 3.24 g Na_2SO_4, 0.5 g $CaCl_2 \cdot 2H_2O$ and 0.9 g KCl. To a liter of this mixture, 5 ml of modified Wolfe's mineral elixir (1, 2), 0.2 ml 1% aqueous resazurin and 0.3 g NH_4Cl were added, and the pH was adjusted to 7.0. A total of 1.6 g of Bacto agar (Difco Laboratories, Detroit, MI) was then added, after which the medium was autoclaved.

After autoclaving, the following ingredients were added as sterile stock solutions (per liter) in the five media described above: 0.5 ml of vitamin solution (3), 2.8 ml of 0.5 M $KHPO_4$ buffer (pH 7.0), 3 ml of 10 mM $FeCl_2 \cdot 4H_2O$ (in 0.02 N HCl), 1.8 ml of 0.8 M sodium bicarbonate ($NaHCO_3$) solution and 10 ml of a filter-sterilized, freshly made, 4% neutralized cysteine • HCl • 2H2O solution. In the medium of strains MC-1, MMS-1 and SS-5, 3 ml of 40% sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) was added while the medium of strains SS-1 was supplemented with 5 ml of neutralized 100 mM sodium sulfide ($Na_2S \cdot 9H_2O$). The media were then dispensed into sterile, screw-cap glass tubes at ~80% of their volume and incubated at room temperature for several hours to solidify and to allow the oxygen gradient to form, as evidenced by the presence of a pink (oxidized) zone near the surface and a colorless (reduced) zone in the lower portion of the tubes due to the redox indicator resazurin.

Strains PR-1, PR-2 and PR-3 were isolated from samples collected from the same site at the Pointe Rouge Marina in Marseille, France. Strain SS-1 was isolated from sample collected at Salton Sea, California. Samples contained sediment together with the interface seawater, with a ratio of 1:2. Magnetotactic cells were magnetically concentrated by placing the south pole of a magnetic stirring bar next to sample bottles at the sediment–water interface for ~30 min, and then further purified using the capillary magnetic racetrack technique (4). Cells were used as inocula in semisolid, oxygen-gradient, enrichment media described above. Axenic cultures were obtained by dilution to extinction three times in succession in semisolid growth media.

Determination of 16S rRNA and phylogenetic analysis

16S rRNA genes were amplified using bacteria specific primers 27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-TACGGHTACCTTGTTACGACTT-3' (5). PCR products were cloned into pGEM-T Easy Vector (Promega Corporation, MadisonWI, USA) and sequenced (GATC Biotech AG, Germany). Alignment of 16S rRNA was performed using CLUSTAL W multiple alignment accessory application in the BioEdit sequence alignment editor (6). Phylogenetic trees were constructed using MEGA version 5 (7) applying the neighbor-joining method (8). Bootstrap values were calculated with 100 replicates.

Aggregate formation

About one hour after the transfer of the MTB in the capillary, a low proportion of cells stops swimming and aggregates in the band at the oxic-anoxic interface. These aggregates were

observed for almost all species throughout the entire depth of the capillary (Fig. S5). This behavior is likely due to the attachments of the cells to the walls of the capillary or to each other through the contact of their flagella or pili, as previously reported for *Magnetococcus marinus* (3).

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Table S1: Parameter values used in the numerical calculations.

Parameter	**Value**	**Source**
Swimming speed v	20 μm × s^{-1}	This study
Switching rate (k_{RL} and k_{LR}), basal value	1/6 s^{-1}	(9)
Switching rate (k_{RL} and k_{LR}), increased value	1 s^{-1}	(9)
Oxygen consumption rate κ	0.01 fmol/min/cell	(10)
Oxygen concentration at which consumption is half maximal (c_a)	0.75 μM	(10)
Oxygen concentration at the open end	200 μM	This study
Preferred oxygen concentration (c_{opt})	2.5-3.5 μM	This study and (10)
Oxygen diffusion coefficient (D)	2100 $\mu m^2 \times s^{-1}$	(11)
Total number of bacteria	10^6	See text

TABLE S2 Speed motility (in µm/s) of the cells swimming in the anoxic or oxic zone surrounding the microaerotactic band. Cells swimming from the band toward the anoxic zone (purple), from the anoxic zone toward the band (light blue), from the band toward the oxic zone (red), and from the oxic zone toward the band (dark blue). Table shows the average velocities of eleven MTB depending on their swimming direction and their position compared to the microaerotactic band. Arrows indicate the significant increase (green), decrease (red) or stagnation (yellow) of the speed motility between the – and + directions in the oxic and anoxic zones.

Strain	-	+	-	+
LM1	18,6 ± 0,9	12,1 ± 1	20,7 ± 1,5	29,2 ± 4,6
MC1	54 ± 8,1	54,5 ± 5,1	119 ± 13,6	88,8 ± 11,8
MSR1	13,3 ± 1,1	13,5 ± 0,6	23,3 ± 2,9	15,3 ± 1,9
MV1	7,8 ± 1,3	5,9 ± 1	8,5 ± 2,3	3,6 ± 1,3
PR1	21 ± 3	20,1 ± 3,1	50,5 ± 5,3	35 ± 3,5
PR2	8,4 ± 2,1	6,1 ± 1,2	23,7 ± 3,6	14 ± 1,5
PR3	90,8 ± 7,2	81,7 ± 4,7	109,9 ± 13,2	94,9 ± 6,6
RS1	20,5 ± 1,8	22,1 ± 1,8	n.a. --	n.a. --
SS1	30,6 ± 14,8	40,8 ± 8,7	111,6 ± 21	93,9 ± 19,7
SS5	35,7 ± 2,9	24,7 ± 0,9	32,1 ± 2,2	29,8 ± 1,5
UT4	15,9 ± 1,3	14,3 ± 1	25,9 ± 1,4	23,1 ± 0,9

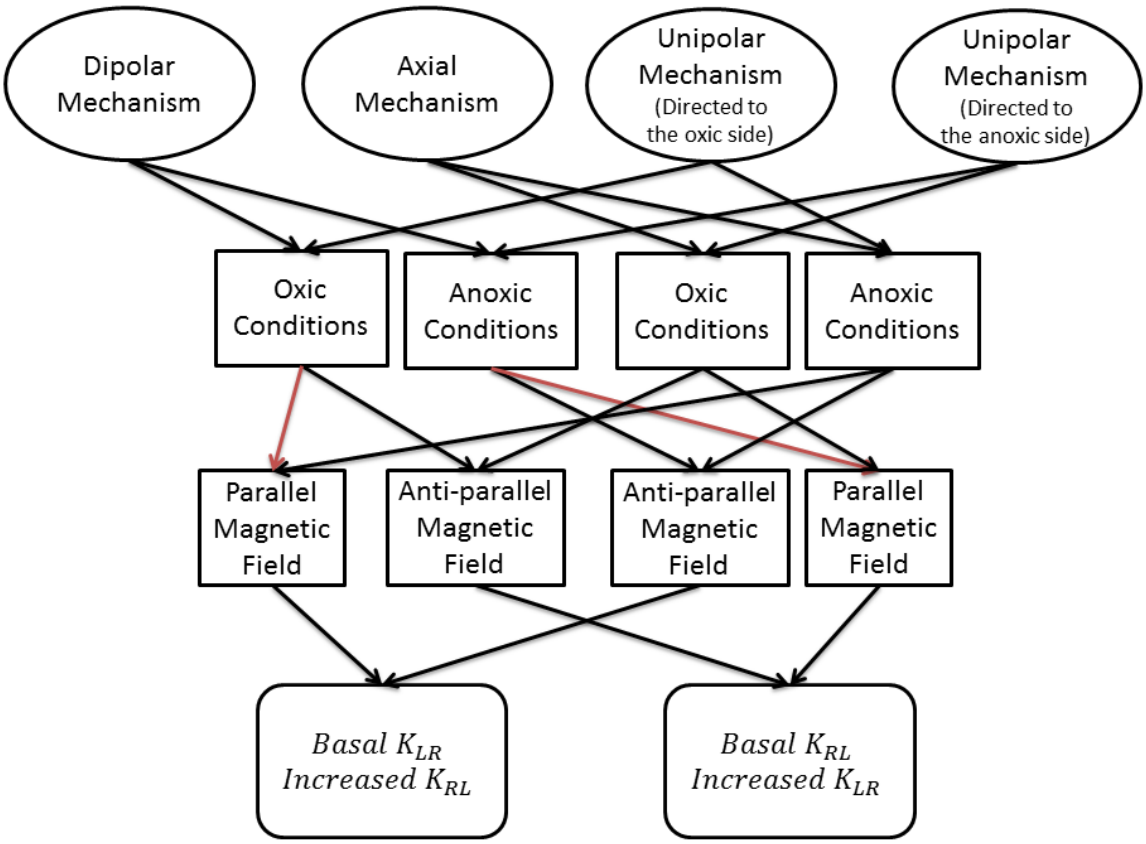

FIGURE S1 Diagram demonstrating how the switching rates for each mechanism were chosen. Red arrows represent paths that lead the cells to swim toward the wrong direction.

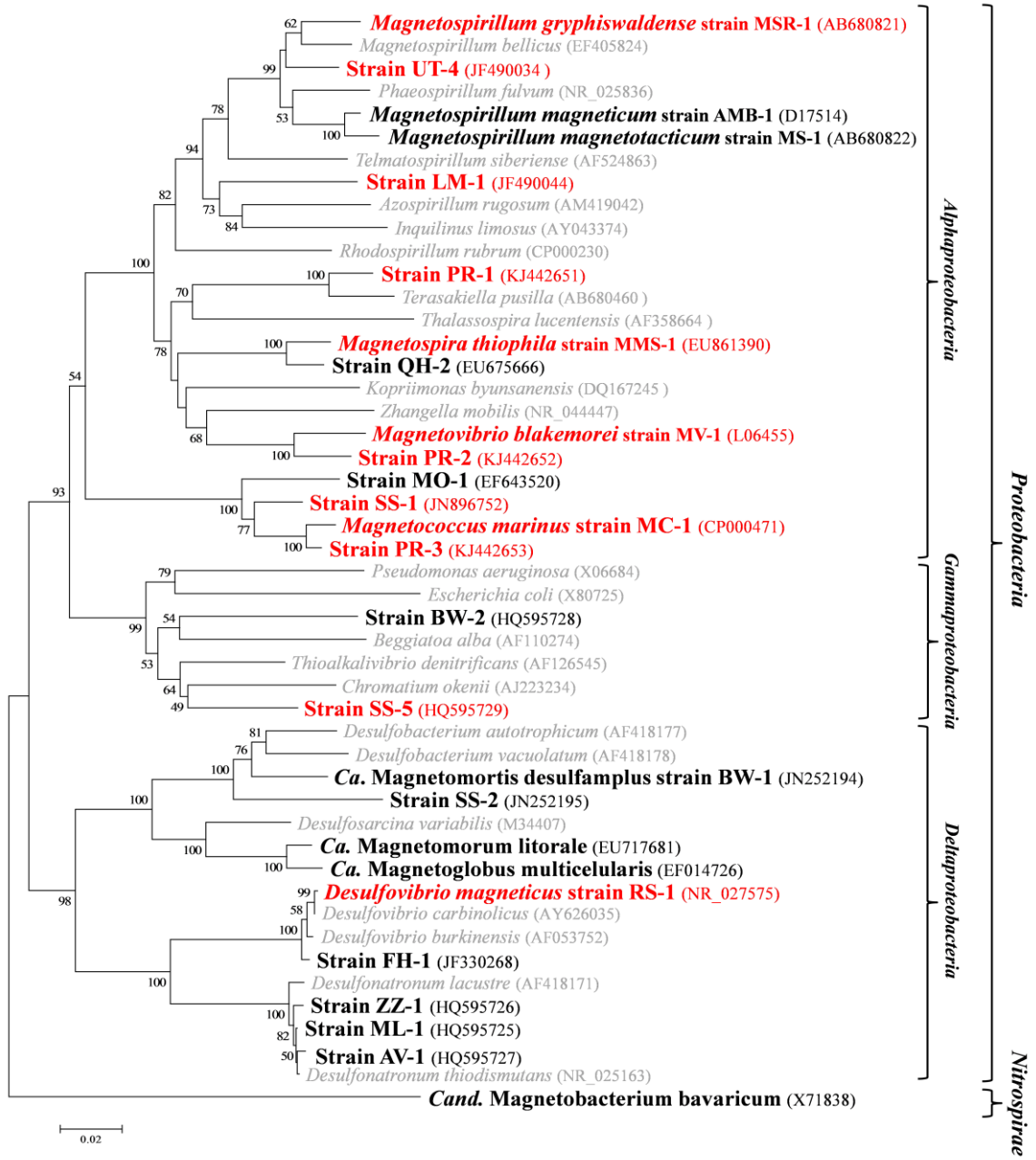

FIGURE S2 Phylogenetic positions of magnetotactic bacteria under study. Phylogenetic tree, based on 16S rRNA gene sequences using the neighbour-joining algorithm, showing the positions of the MTB under study (red), others MTB (black) and related non-magnetotactic bacteria (grey). Bootstrap values (higher than 50) at nodes are percentages of 100 replicates. Accession numbers are given in parentheses. Bar represents 5% sequence divergence.

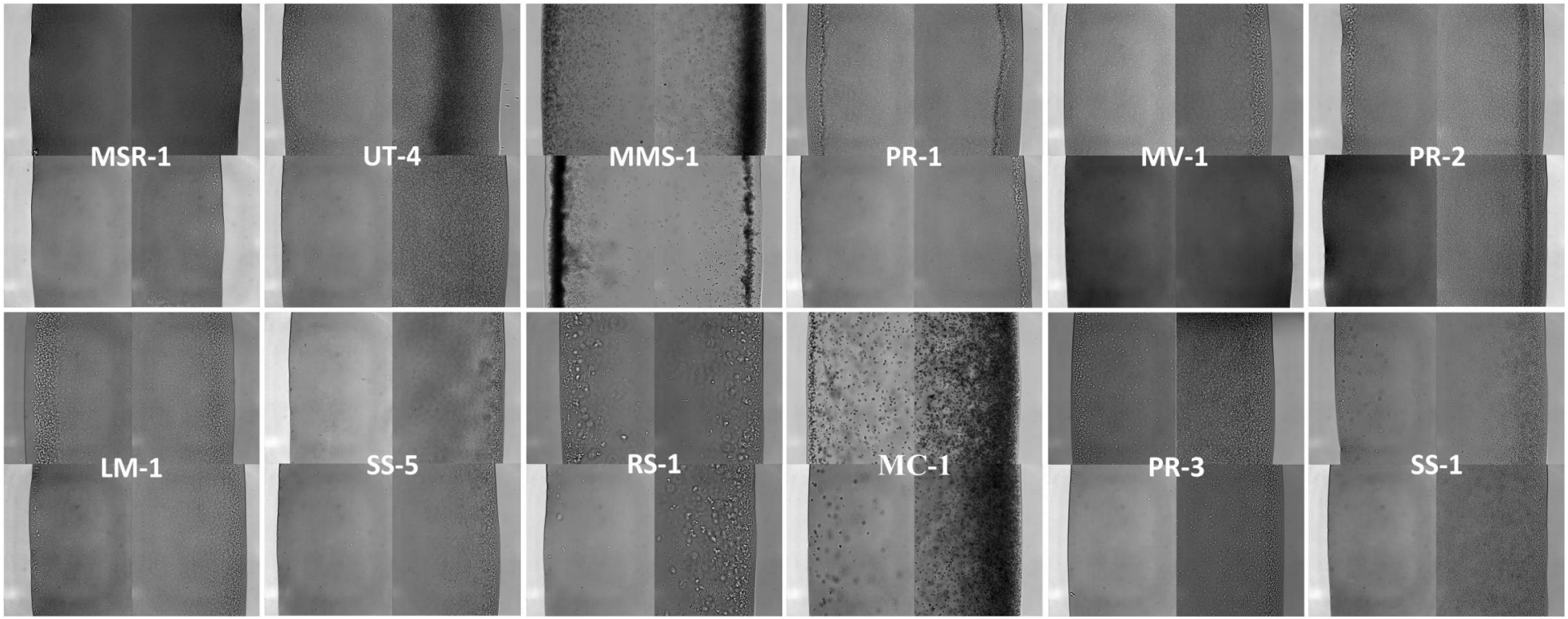

FIGURE S3 Observation of the magnetic behavior of twelve magnetotactic bacteria studied under the light microscope using the hanging drop technique. Before magnetic separation (top half of each panel), a majority of cells aggregate at the north side of the drop (upper right quadrant of each panel) whereas after magnetic separation (lower half of each panel) few or no south seeking cells remain, except for strain MMS-1 whose magnetic polarity is affected by the light of the microscope.

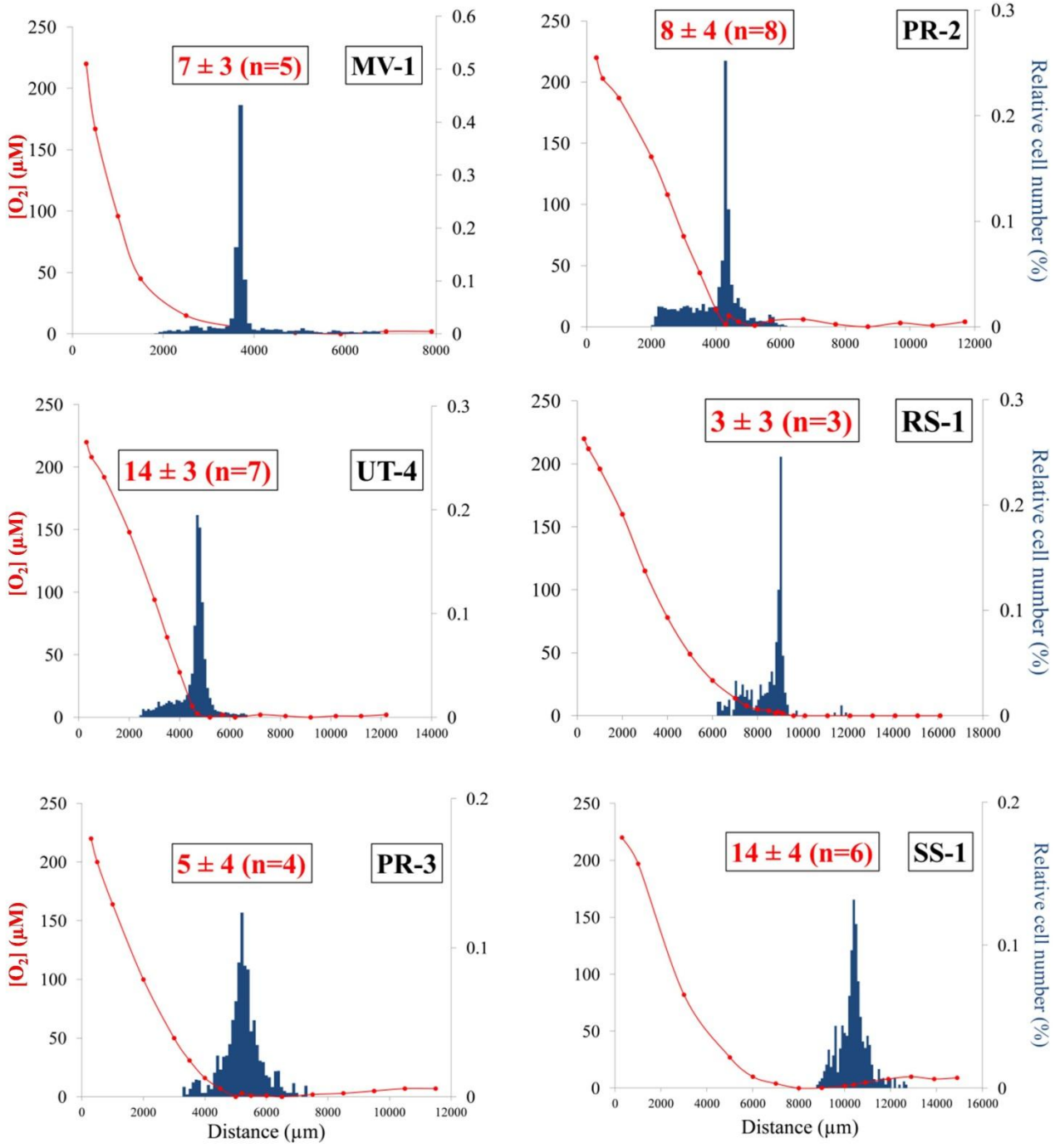

FIGURE S4 Relative cell distributions (blue histograms) of the twelve different magnetotactic bacteria under study in oxygen gradients (red circles). Each graph was made from a single microcapillary. Oxygen concentrations were obtained from one measurement at different positions along the capillary. In red is the value of the oxygen concentration (in µM) in the center of the aerotactic band (n represents the number of capillaries used to measure the average oxygen concentration at the band; see Materials and Methods for the error calculation). The relative number of cells were measured every 100 µm from triplicate counts. The same capillary was also used to measure the oxygen concentrations. For strain UT-4, the distribution of cells were determined from a total of 17,668 cells; for strain MV-1 from 2,252 cells; for strain PR-2 from 4,489 cells; for strain RS-1 from 265 cells; for strain PR-3 from 732 cells; and for strain SS-1 from 462 cells.

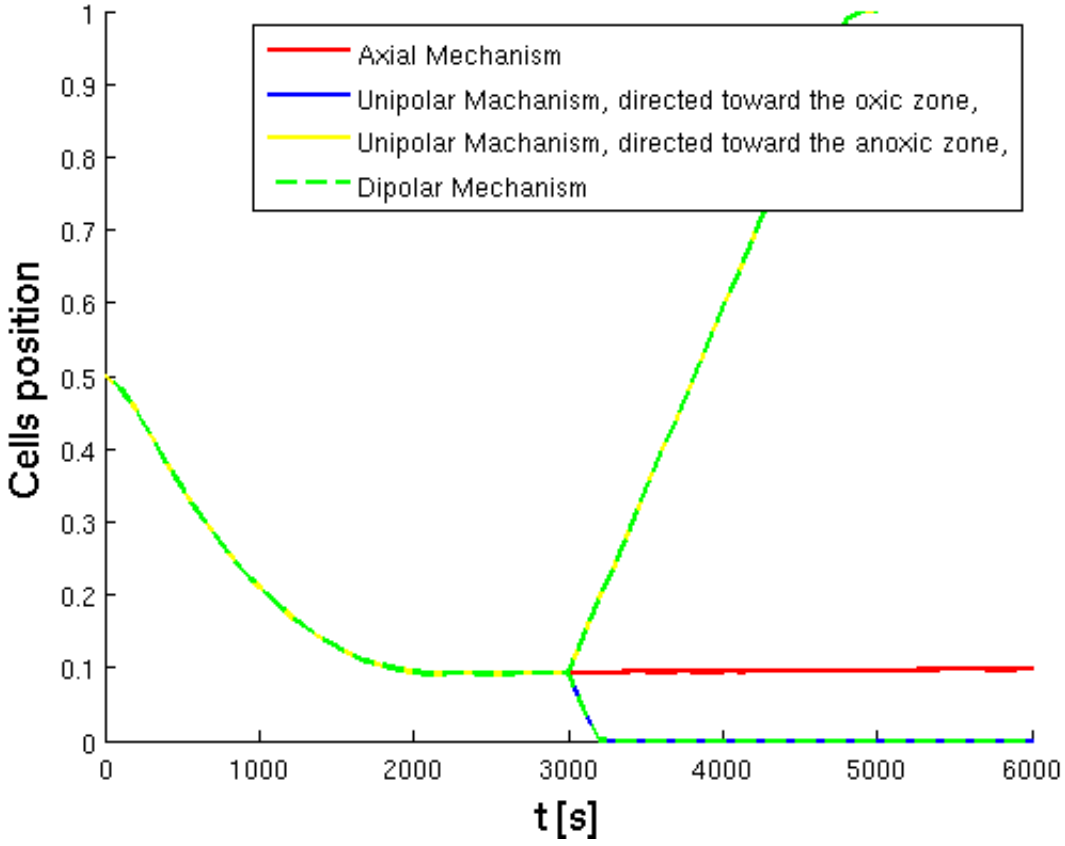

FIGURE S5 Relative position of the bacteria in the capillary while the band is formed and after the magnetic field is reversed (after 3000 s) for the different mechanisms. For the dipolar mechanism, after the reversal of the magnetic field, the positions of both bands are shown.

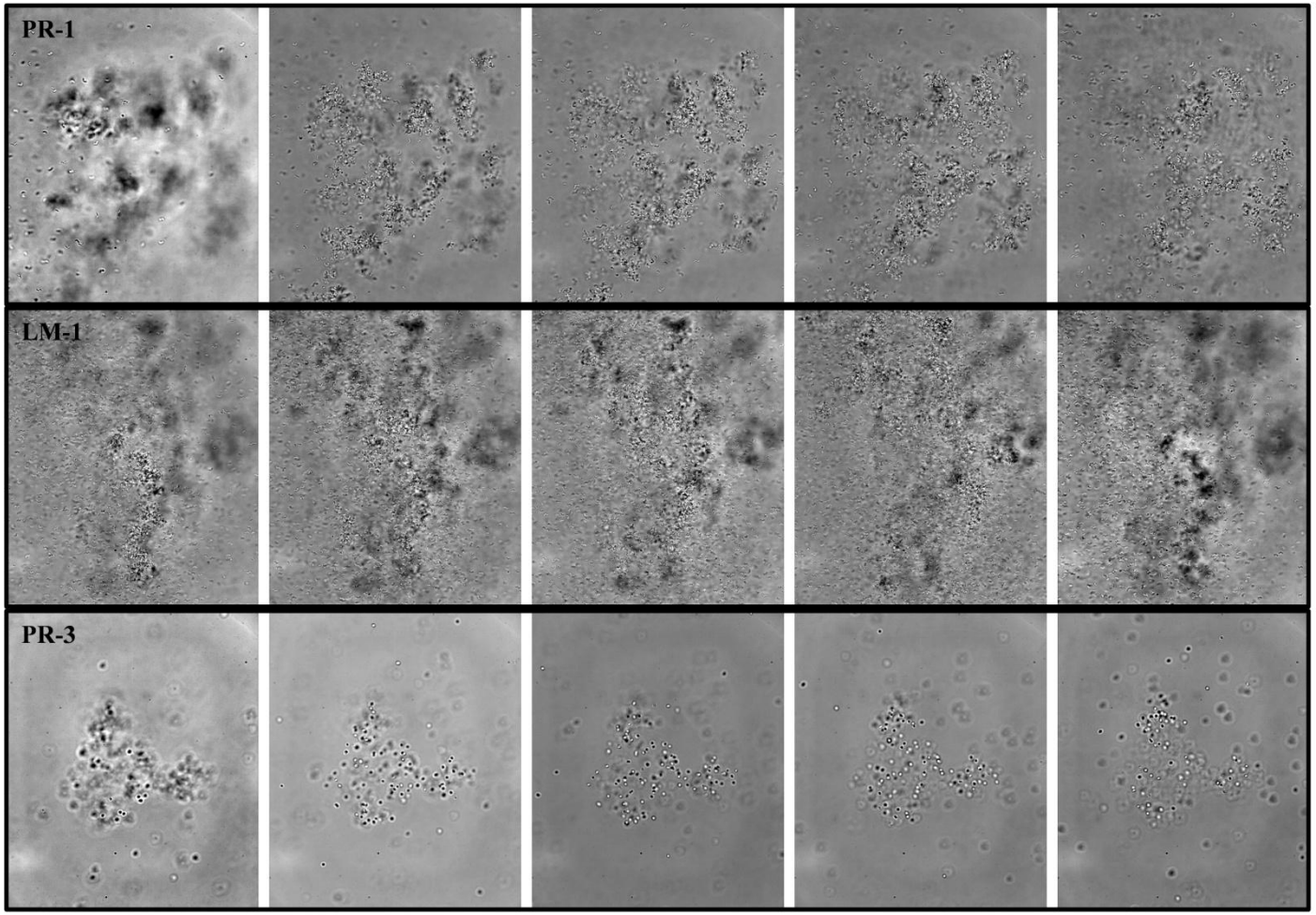

FIGURE S6 Formation of aggregates by strains PR-1, LM-1 and PR-3 at the oxic-anoxic interface, one hour after the formation of the microaerobic band. In each frames the different panels are separated by a distance of 5 µm in the Z axis.

MOVIE S1 Cells of strain SS-5 moving in the anoxic zone of the aerotactic band. Cells continue swimming back and forth along the magnetic field through the band. Once they reached the regions of too high or too low oxygen concentration, cells reversed their swimming direction without reorientation of the cell body.