Environmental Microbiology (1999) **1**(6), 489–494

Aerotaxis in *Desulfovibrio*

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Summary

Aerotaxis of two sulphate-reducing bacteria, the freshwater strain *Desulfovibrio desulfuricans* CSN (DSM 9104) and the marine strain *Desulfovibrio oxyclinae* N13 (DSM 11498), was studied using capillary microslides, microscopy and oxygen microsensors. The bacteria formed ring-shaped bands in oxygen diffusion gradients surrounding O_2 bubbles, which were placed into anoxic sulphate-free cell suspensions in capillary microslides. The radial expansion of the oxic volume by diffusion was stopped by aerobic respiration. Bands were formed by cells avoiding high O_2 levels near the O_2 bubble, as well as by cells entering from the surrounding anoxic zone. At the inner edge of the bands, O_2 levels of up to 20% air saturation (50 μM O_2) were found, while the outer edge always coincided with the oxic–anoxic interface. Ring diameters and O_2 concentrations at the inner edge of the band depended on the cell density and the strain used in the suspension. Band formation did not occur in the absence of an electron donor (5 mM lactate) or when N_2 gas bubbles were used. Both strains were highly motile with velocities of ≈ 32 μm s^{-1} during forward runs, and 7 μm s^{-1} during backward runs respectively. Within the bands, cells moved in circles of about 20 μm diameter, while cells outside the band exhibited straighter or only slightly bent traces. It is concluded that the capacity of respiration at high rates and the positive and negative aerotactical responses of *Desulfovibrio* provide an efficient strategy for removing O_2 from the habitat in situations where sufficient electron donors and high cell densities are present.

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Introduction

Chemotaxis is usually regarded as a mechanism allowing motile bacteria to find sites of favourable environmental conditions or, alternatively, to avoid regions with unfavourable conditions. Thus, strict anaerobes are repelled by oxygen (Beijerinck, 1895; Armitage, 1997). Sulphate-reducing bacteria (SRB) have traditionally been classified as strict anaerobes. However, several studies have shown that high numbers of SRB occur in oxic or periodically oxic zones of sediments and microbial mats (Laanbroek and Pfennig, 1981; Cohen, 1989; Bak and Pfennig, 1991; Jørgensen and Bak, 1991; Fründ and Cohen, 1992; Visscher *et al*., 1992; Sass *et al*., 1996; 1997; Teske *et al*., 1998). Their activities in these environments are not fully understood. While sulphate reduction in oxic natural environments has been reported in the literature (Canfield and Des Marais, 1991, Visscher *et al*., 1992; Jørgensen, 1994), in pure cultures, sulphate reduction is inhibited by oxygen (Marschall *et al*., 1993). However, many SRB can respire with oxygen (Dannenberg *et al*., 1992) and switch to aerobic respiration if oxygen is present (Krekeler and Cypionka, 1995). Several strains of *Desulfovibrio* show aerobic respiration rates that are 10 times higher than their sulphate reduction rates and even exceed the respiration rates of aerobic bacteria (Kuhnigk *et al*., 1996). The reduction of O_2 enables ATP production (Dilling and Cypionka, 1990). However, the high potential respiration rates indicate that this capacity has the function of consuming oxygen in order to re-establish the conditions for anaerobic growth rather than conserving energy.

SRB do not grow in homogeneously aerated cultures (Dannenberg *et al*., 1992; Marschall *et al*., 1993; Sass *et al*., 1996; Krekeler *et al*., 1997a). In stratified systems, however, different behavioural patterns have been observed. In artificial oxygen–sulphide gradients, SRB grew in bands just below the oxic–anoxic interface (Cypionka *et al*., 1985; Marschall *et al*., 1993). Under a headspace with defined oxygen concentrations, *Desulfovibrio vulgaris* was reported to grow in bands at oxygen concentrations below 0.05% (or 6 μM O_2; Johnson *et al*., 1997). In cyanobacterial mats, cell numbers of SRB in the upper layers varied with daytime and O_2 concentration, indicating migration of SRB (Krekeler *et al*., 1997a; Teske *et al*., 1998). Pure culture studies of SRB revealed two other types of responses to O_2, aggregation of cells and band formation (Krekeler *et al*., 1997a, Johnson *et al*., 1997). While aggregation appears as an attempt

to avoid oxygen exposure, the formation of bands of high bacterial cell density indicates a more complex behavioural response of some SRB towards O_2.

In this study, we have characterized the motility, aerotaxis and band formation of two *Desulfovibrio* strains in relation to experimental O_2 diffusion gradients. Besides microscopy, the band formation and O_2 distribution were studied by image analysis and O_2 microsensors.

Results

Band formation and oxygen diffusion

The bacteria formed ring-shaped bands around air or oxygen bubbles within a few minutes (Fig. 1A). Inside the band, the cell suspension turned pink by the oxidized form of the added redox indicator reazurin, while the suspension outside the band remained colourless. Band formation depended on the availability of an electron donor and oxygen. No accumulation was observed in suspensions without lactate. The ring diameter increased with time and depended on the oxygen concentration in the bubble. Rings were smaller around air than around oxygen bubbles. In control experiments with N_2, no band formation was observed (Fig. 1A).

The size of the oxic volume depended on the density of the cell suspension (Figs 1B, 2 and 3). In control experiments without cells, oxygen diffusion caused a rapid expansion of the oxic volume to the edges of the capillary (Fig. 3). The measured expansion corresponded well with that calculated for oxygen diffusion through water from a cylindrical bubble at 20°C. With bacterial suspensions, the expansion of the oxic volume slowed down drastically after a few minutes (Fig. 2) as a result of aerobic respiration. The oxygen in the bubble was completely consumed overnight, and only a tiny bubble, probably consisting of nitrogen, was left, while the redox indicator resazurin was colourless. Both strains studied behaved similarly in these experiments. However, in suspensions of *D. oxyclinae*, the oxic volumes became approximately twice as large as in suspensions of *D. desulfuricans* (not shown).

If sulphate was added to the cell suspensions, the bands developed closer to the oxygen bubble. Sometimes double bands and sulphur precipitations were observed. Obviously, sulphate was reduced, and the sulphide formed reacted with oxygen chemically or catalysed by the cells.

Oxygen concentrations and respiration rate

By means of the oxygen microelectrode, it was found that oxygen was always completely consumed at the outer edge of the bands, while the O_2 concentration at the inner edge depended on the cell density (Fig. 3) and the strain investigated. With *D. desulfuricans*, the inner edge of the band was found at about 50 μM O_2 (20% air saturation) at an optical density (OD) of 10 (corresponding to 2 mg dry mass $\times$ ml^{-1} or 5×10^9 cells ml^{-1}), and 30 μM O_2 at an OD of 3. With *D. oxyclinae*, the oxygen concentrations at the inner edge of the bands were about 30% of this at the same cell densities.

In order to quantify the accumulation of the bacteria in the band, the turbidity of the cell suspension across the ring was measured using an optic fibre of 50 μm diameter (Fig. 4). At the anoxic side of the band, a decrease in density was observed compared with the mean OD in the capillary. Obviously, part of the band-forming cells came from the anoxic zone.

Motility of single cells

Single cell movements were recorded as light traces on darkfield microphotographs taken with an exposure time of 1 s. Forward runs (on average 32 μm s^{-1}) were indicated by thin traces. Backward runs (7 μm s^{-1}) gave thicker and shorter traces. Cell reversal typically gave an acute angle and resulted in a random walk. Tumbling, as known from bacteria with peritrichous flagellation, was not observed. In homogeneously anoxic suspensions (Fig. 1D), we observed mainly straight or slightly curved lines. Only a few cells (21 out of 92 traces analysed) made a reversal within the exposure time of 1 s, indicating a mean running time of several seconds.

In oxygen gradients around tiny air bubbles, which were enclosed below the coverslip (Fig. 1C), the cells changed their moving behaviour. Circular traces became more frequent within the band. The circles were about 20 μm in diameter (Fig. 1E). The speed appeared unchanged, while the number of reversals was difficult to determine because of the high cell density in the bands.

Discussion

Our study shows complex patterns of chemotactic behaviour of sulphate-reducing bacteria involving both positive and negative responses to oxygen.

The observation that a clear zone developed near the oxygen bubbles and that the bands were formed at some distance from them shows that high oxygen concentrations function as a repellent. This is not surprising, as even many aerobic bacteria show similar behaviour towards pure oxygen (Armitage, 1997). The mechanisms of negative aerotaxis are not yet well understood in aerobic (as in sulphate-reducing) bacteria. It appears, however, that different receptors are involved in positive and in negative aerotaxis in *E. coli* (Grishanin and Bibkov, 1997).

We made the unexpected observation that the SRB did not swim into the anoxic zone as expected for obligate

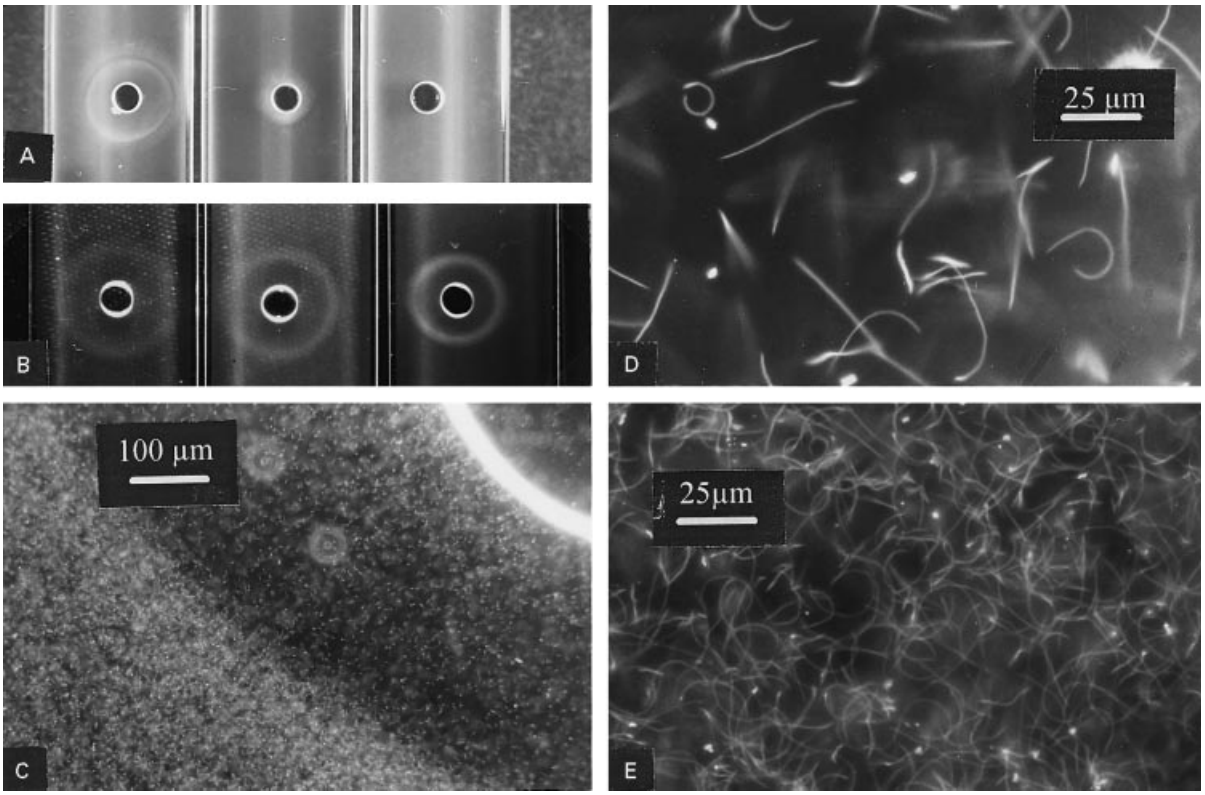

Fig. 1. Band formation and motility patterns of *Desulfovibrio.*
A and B. Bands formed by *Desulfovibrio oxyclinae* N13 around gas bubbles (5 μl) in capillary microslides. The assay contained 5 mM lactate but no sulphate.
A. Comparison of band formation around oxygen, air and nitrogen (from left). The picture was taken after 1 h. The cell suspensions had an optical density (OD_{436}) of 7 (about 1 mg protein ml^{-1}).
B. Band formation at different cell densities after 30 min. The cell suspension had optical densities of 3, 5 and 10 (from left).
C–E. Band formation and motility patterns of single cells of *Desulfovibrio desulfuricans* CSN. The cells were transferred to a coverslip directly from a growing culture and observed by darkfield microscopy.
C. The cells accumulated around a tiny air bubble enclosed below the coverslip. The picture was taken after 2 min at an exposure time of 0.2 s with 125-fold magnification.
D and E. Traces of single cells at 500-fold magnification within an exposure of 1 s (D) in the anoxic part of the cell suspension and (E) in the oxic zone of the gradient.

anaerobes. Instead, they accumulated in the oxic zone at concentrations of up to 20% air saturation. We even observed that part of the cells in the bands came from the outer anoxic zone into the band. Thus, the reaction was not aerophobic. However, a classification as positive aerotaxis seems not to be justified, as discussed below.

Accumulation in bands depended on the availability of an electron donor. This has important consequences. The electron donor allows for the reduction of oxygen. Oxygen consumption results in a steeper slope of the oxygen gradients and higher oxygen diffusion rates into the band. Furthermore, the cells can gain energy by respiration. Last but not least, they have the chance finally to re-establish anoxic conditions. It should be pointed out that only cells in the oxic zone can do this, while cells that have moved to the anoxic part have no opportunity to remove oxygen.

Altogether, the bacterial behaviour fits perfectly with a strategy that is directed to remove oxygen from the environment in order to establish anoxic conditions for growth. Also, the observation that concentrated cell suspensions accumulated at higher oxygen concentrations than diluted ones is in accordance with this. Energy conservation by aerobic respiration cannot be coupled to growth but might be important for motility, as discussed below.

Some of our observations might have rather simple explanations without the assumption of a true bacterial strategy. First, oxygen-sensing mechanisms are known for SRB. *Desulfovibrio vulgaris* possesses a haem-containing protein that senses the redox potential of the environment and might also provide information on the oxygen concentration (Fu *et al*., 1994). Secondly, when oxygen is present, it is always preferentially reduced by

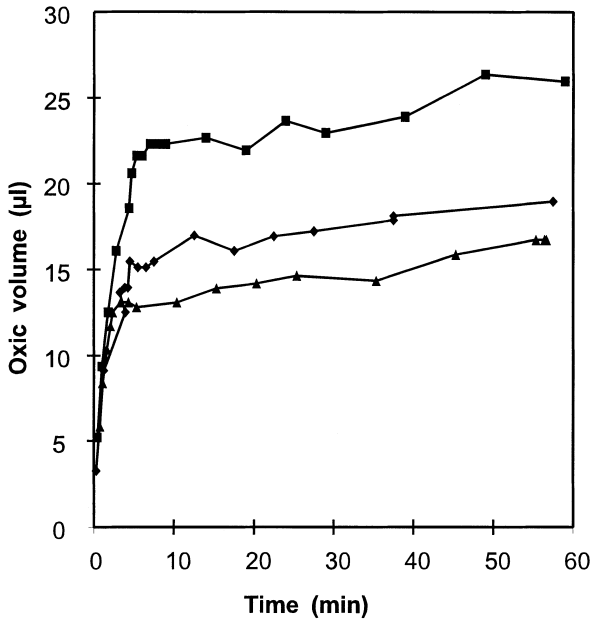

Fig. 2. Development of the oxic volume around oxygen bubbles independently of the cell density. Washed cells of *Desulfovibrio desulfuricans* CSN were incubated in buffer with 5 mM lactate, at OD 3 (■), 5 (♦) and 10 (▲). The oxic volumes were calculated from the diameters of the pink zone around the oxygen bubbles indicating oxidized resazurin (100 µM).

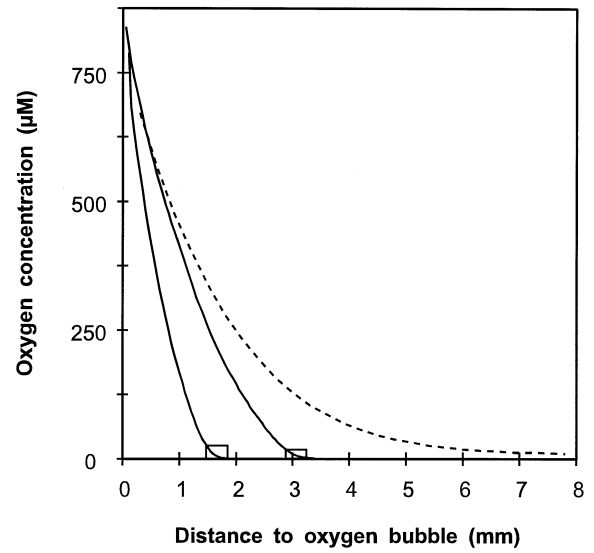

Fig. 3 Oxygen profiles measured by means of an oxygen microelectrode around oxygen bubbles in microslide capillaries 20 min after injection of an oxygen bubble. The assays contained 5 mM lactate and cells of *Desulfovibrio desulfuricans* CSN at $OD_{436} = 10$ (left line) or 3 (middle). The dashed line shows diffusion of oxygen in a control assay without cells. Locations of the bacterial bands are indicated. The oxygen concentration at the inner edge of the band was 21% air saturation at an OD of 10 and 13% at an OD of 3.

the strains studied here (Krekeler and Cypionka, 1995; Krekeler *et al*., 1997a). The cells appear to be unable to regulate the aerobic respiration and often show rates that are much higher than their sulphate reduction rates and even higher than the respiration rates of aerobic bacteria. Although aerobic respiration is not coupled to growth, the electron flow results at least partially in proton translocation and energy conservation (Dilling and Cypionka, 1990; Fitz and Cypionka, 1989, 1991). The arising electrochemical potential could change the motility pattern, as has been shown for *E. coli* and *Salmonella typhimurium* (Shioi *et al*., 1988). In our case, an unknown mechanism forces the cells to move in circles as observed. This, of course, will only happen if an electron donor for the reduction of O_2 is available. Furthermore, this could explain how cells entering the ring from outside by chance were trapped in the oxic zone. In contrast to movement by random walk and random reversal, the circular movement would not allow them to escape from the oxic zone.

Although the freshwater strain showed a higher oxygen tolerance than the strain isolated from the hypersaline mat that is regularly exposed to oxygen supersaturation (Krekeler *et al*., 1997b), the strains studied here showed similar behavioural patterns. The strategy of oxygen removal, enabling the cells to grow anaerobically later on, was observed in growth experiments with SRB isolated from the oxic–anoxic boundary layer of an oligotrophic lake (Sass *et al*., 1997). Quite different behaviour was reported for *D. vulgaris* by Johnson *et al*. (1997). They

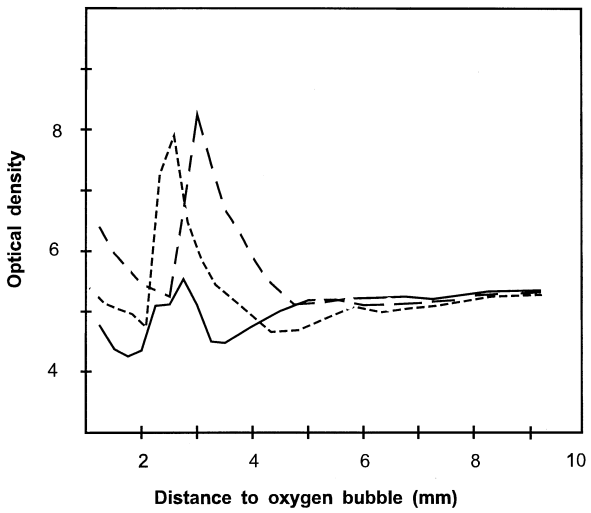

Fig. 4. Optical densities around an oxygen bubble in a suspension of *Desulfovibrio desulfuricans* CSN with an OD_{436} of 5 (about 1 mg dry mass ml^{-1} or 2.5×10^9 cells ml^{-1}), containing 5 mM lactate, after 10 min (solid line), 20 min and 40 min (short dashed and long-dashed lines respectively). OD was measured by means of an optic e (50 µm) across a capillary microslide 1 mm thick.

found that *D. vulgaris* did not move in circles but switched to a higher swimming speed in the presence of oxygen. The cells were reported to grow in bands at low oxygen concentrations (which were, however, extrapolated without measurements by microsensors). Thus, it appears that there is more than one strategy for responding to oxygen in SRB. It should be mentioned that the assays studied so far did not contain any sulphur compounds, and sulphate reduction or oxidation of sulphide were precluded. Interpretation of preliminary experiments with gradients of sulphide and oxygen was complicated by the formation of elemental sulphur. Further investigations, including microscale measurements of both oxygen and sulphide, are in progress.

Experimental procedures

Organisms and cultivation

The freshwater strain *Desulfovibrio desulfuricans* CSN, DSM 9104, originally isolated from anoxic sludge (Cypionka, 1989), and the marine strain *Desulfovibrio oxyclinae* N13, isolated from a cyanobacterial mat from Solar Lake (Krekeler *et al*., 1997a,b), were grown at 30°C with lactate (20 mM) and sulphate (20 mM) in mineral medium, as described by Cypionka and Pfennig (1986) and Krekeler *et al*. (1997b). The redox indicator resazurin (0.25 mg l^{-1} or 1 μM) was added to the medium and reduced with traces (< 30 mg l^{-1}) of sodium dithionite until it was colourless.

Before experiments, freshly grown cultures were purged with CO_2 to remove H_2S and harvested by centrifugation (15 min at 14 000 × *g*). Cells were washed and resuspended in N_2-flushed buffer, pH 7.0, containing 10 mM KH_2PO_4, 0.1 mM sodium EDTA and, for the marine strain, 350 mM NaCl.

Experimental set-up

Diffusion of oxygen in cell-free buffer and bacterial band formation was studied in capillary microslides (100 × 10 × 1 mm; Vitro Dynamics). Buffer and cell suspensions were reduced with traces of dithionite and filled into the capillary using a syringe. Then, gas bubbles (pure oxygen, air or nitrogen) of 5 μl volume were placed into the middle of the capillary using a Hamilton syringe.

Diffusion of oxygen and band formation were documented by macrophotography in assays with resazurin (50–200 μM). Alternatively, oxygen concentrations within the diffusion gradients and the bands were determined by means of a Clark-type oxygen microelectrode (Revsbech, 1989). In those assays, resazurin could be omitted. The microelectrode was mounted on a motorized micromanipulator (Märtzhäuser). The tip diameter of the microelectrodes was < 10 μm, and the stirring sensitivity was < 2%. In order to fit into the capillary, the electrode casing had an outer diameter of < 1 mm over the outermost 4–5 cm. The microelectrode was connected to a pA meter, and measurements were recorded on a strip chart recorder as well as on a personal computer equipped with a data acquisition card. Positioning of the micromanipulator and data acquisition were controlled by custom-made measuring software. The microelectrodes were calibrated linearly from readings in medium saturated with O_2, air and N_2.

In order to quantify the accumulation of the cells in bands, the OD across the capillary was measured using an optic fibre (50 μm ø) coupled to a diode array spectrometer (PC1000; Ocean Optics). The capillaries were moved through the light beam with a micromanipulator. The turbidity was measured by integrating the absorption over a range from 500 to 750 nm and compared with the absorption of cell suspensions of known density.

Motility of single cells was documented by means of dark-field microphotography and microphotography (Leitz DM RBE and Leica Photoautomat Wild MPS 48/52). A drop of freshly grown bacteria was transferred directly from the growth medium and sealed under a coverslip with paraffin. Assays with and without included air bubbles were compared. During exposure times of 1–4 s in the dark field, moving bacteria could be detected as light traces. The lengths of the traces were measured using a map measurer after magnified projection of the photos.

Acknowledgements

The authors wish to thank Christian Eder, Irmgard Wolke, Uta Grünert and Daniel Krekeler for helpful advice during this project. This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Cy 1/6-3). M.K. acknowledges support from the Max-Planck-Gesellschaft and the Danish National Science Research Council.

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