"*Candidatus* Ovobacter propellens": a large conspicuous prokaryote with an unusual motility behaviour

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Abstract

A new type of bacterium "*Candidatus* Ovobacter propellens" is described. It is a large ovoid species with a number of unusual features including special intracellular membrane systems and a huge flagellar tuft consisting of about 400 flagella. "Ovobacter" lives in oxygen gradients in the surface layer of sulphidic marine sediments at an O_2-tension of about 0.5% atmospheric saturation. It swims continuously and very fast (up to 1 mm s^{-1}). Its mode of swimming and its chemosensory behaviour towards O_2 are described in detail.
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1. Introduction

Steep O_2-gradients in surface layers of marine sediments harbour a variety of unusual bacteria. These include large sulphur oxidising bacteria like *Thiovulum* and *Beggiatoa* as well as several other types of bacteria. Common to all these organisms is a distinct preference for a given O_2-tension and that they display chemosensory motile behaviour that allow the organisms to form narrow bands at the preferred O_2-tension [1–7]. Some of these bacteria are unusually large and thus belong to the category of "big bacteria" in the sense of Schulz and Jørgensen [8].

Here, we describe a novel type of a large bacterium that lives in steep O_2-gradients in the sediment surface at O_2-tensions of about 0.5% atmospheric saturation. Special emphasis is on its motile behaviour. The structure of this conspicuous organism is unusually complex compared to other prokaryotes. Its mechanism of swimming also deviates somewhat from what has otherwise been described for prokaryotes and its swim-ming velocity (up to 1 mm s^{-1}) is probably the highest ever recorded for bacteria.

2. Materials and methods

2.1. Materials

Sandy, sulphidic sediments were collected in a shallow bay (Nivå Bay) in the Sound about 25 km north of Copenhagen and in the North Harbour in Helsingør, in both cases at depths of about 0.5 m. In the laboratory the sediments were placed in small aquaria and covered by air bubbled seawater. At intervals, small pieces of Kleenex tissue were buried into the sand to maintain a reducing environment immediately beneath the sediment surface. In this way a reliable source of the bacteria could be maintained for several months. Whitish or greyish bacterial surface films were collected with a pipette as the source for the bacteria. The collected microbial films harboured diverse bacterial biota including sulphur bacteria (mainly *Thiovulum* and *Beggiatoa*), three morphotypes of large spirilla, a *Siderocapsa*-like bacterium, an unnamed vibrioid species [6], various

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smaller bacteria, and the species described in this paper. It was usually possible to find patches on the sediment surface where the latter species dominated. In microscopic preparations the bacteria emerged from detrital particles as the ambient O_2 concentration decreased and eventually the cells formed narrow bands at the oxic–anoxic interface close to the edge of the preparations.

2.2. Morphological studies

Cells were observed living or fixed in OsO_4 vapours using Nomarski Interference microscopy. For electron microscopy cells were fixed in 1% OsO_4 and 5% glutaraldehyde in filtered (0.2 μm) seawater in centrifuge glasses for about 15 min. After rinsing with distilled H_2O, cells were compacted into a pellet in a centrifuge glass and the pellet was then imbedded in a drop of 40 °C, 2% agar. The congealed agar drop was dehydrated in an ethanol series, embedded in epon, and serial sections (thickness about 0.1 μm) were cut with an LKB microtome, stained with uranyl acetate, and observed in a Zeiss EM-900 electron microscope. Photographs of successive serial sections were traced on plexiglass plates with an appropriate thickness to produce 3-D reconstructions of smaller or larger parts of the cells.

2.3. Motility

Swimming cells were recorded with high-speed video (High-Speed CCD-camera, MS-1000, Mega Speed Co-operation, Minnedosa, Canada) using recording-frequencies of 192, 658, or 963 frames per second (time resolution: about 5, 1.5, and 1 ms, respectively) of swimming cells with 20× or 40× objectives and Nomarski interference microscopy. Since white light repels the bacterium, we used red light by placing a red filter in the illumination path. Afterwards, successive frames were displayed on the monitor and positions of cells and the position of the flagellar tuft on individual cells could be marked on a transparent overlay.

2.4. Position and orientation in O_2-gradients

Flat microcapillaries (VitroCom Inc.), with internal height of 0.8 mm and width of 8 mm were filled with material from a microbial film and the capillaries were mounted on an inverted microscope (10× phase objective) fitted with a video camera. When the bacteria had produced a well-defined band at some distance from and parallel to the air–water interface at the end of the capillary, an oxygen microelectrode with a tip diameter of about 10 μm [9] was inserted in steps of 50 μm until anoxic conditions were recorded behind the bacterial band.

3. Results

3.1. Structure of "Ovobacter propellens"

The cells measured 4–5 μm. The overall shape of the cells was ovoid. From one side they appeared ovoid and from a perpendicular view they appeared spherical (Fig. 1). In the anterior end there was a prominent flagellar tuft that was easily visible even at a low magnification. It stretched about 3/4 of the cell length when viewed from the ovoid side, but when viewed from the side where the cells had a circular transect, it appeared narrow; from this angle it could sometimes be observed that the flagella emerged from a longitudinal depression on the cell surface. Superficially, the living, rapidly swimming bacterium appeared more like a small unicellular eukaryote than a prokaryote organism.

The cytoplasm is mainly concentrated in the periphery of the cell giving the impression of a refringent ring around the cell, and staining with DAPI showed that the cell's DNA was concentrated in the periphery of the cell. The cytoplasm surrounded a large central vacuole filled with refringent inclusions. They stained intensively with Sudan black and most likely consisted of polyhydroxyalkanoic acids.

Electron microscopy provided a more detailed picture of the structure of "Ovobacter" (Figs. 2–4). We consider the side carrying flagella as the anterior end and – quite arbitrarily – that the side with the peculiar membrane structures (Fig. 2) as the "ventral" side. The cells had a central depression or groove along most of the anterior end, but it did not extend all the way to the left side. Most flagella were rooted in this depression starting about 1 μm from the left side end forming an uninterrupted flagellar field that extends all the way to the right side. Towards the dorsal side the flagella were only found in the groove, but on the ventral side of the cell, the flagellar field also extended somewhat outside the groove beyond the bordering ridge (Figs. 2, 3(a), and 4(a)). The flagellar tuft always bends backwards.

The tripartite cell wall appeared to be typical of Gram-negative bacteria (Fig. 4(c)). Along the periphery of the cell, a system of interconnected membrane
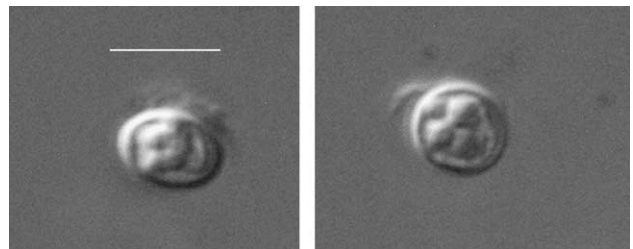

Fig. 1. Light microscopic aspect of Ovobacter propellens (fixed with OsO_4 vapours) seen from the "dorsal" side and from the left side. Scale bar: 5 μm.
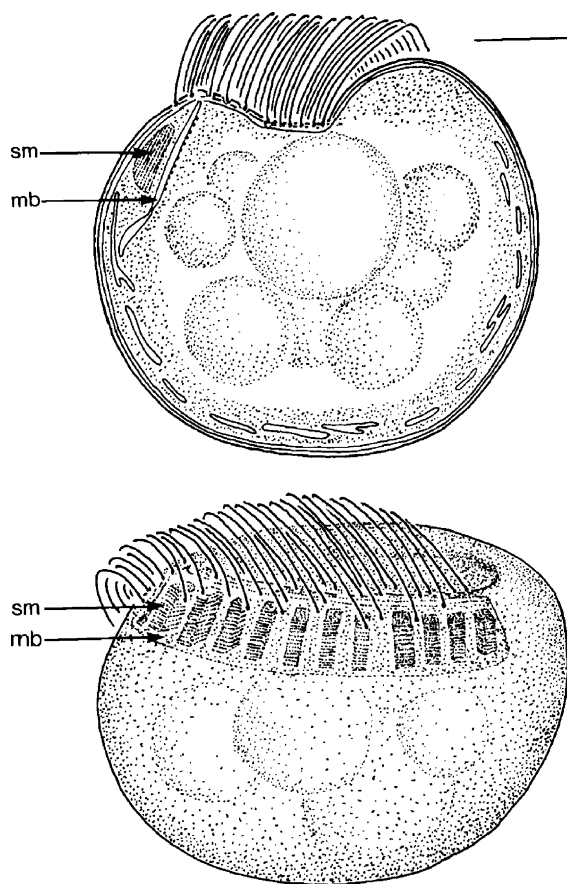
Fig. 2. Schematic presentation of the structure of Ovobacter propellens. Above: transverse section ("ventral" side to the left). Below: the cell seen from the "ventral side" (with the right end of the cell turning to the left in the figure); sm: stacked membranes, mb: membrane band. Scale bar: 1 μm.

channels was found; they represent invaginations of the inner cell membrane. The lumen of these channels are connected to a narrow membrane band or fold that followed the ventral border of the flagellar groove; towards the right end of the cell the band turned dorsally and so also surrounded the right end of the groove. The proximal side of the membrane band was coated with ribosomes. Distal to this band a series of 12–13 box-shaped, electron dense organelles were placed at regular intervals, they consist of stacked membranes. Each of these stacks had an electron dense dark layer passing through their centre perpendicularly to the membranes (Figs. 3(a)–(d) and 4(b) and (c)).

The mean distance between adjacent flagella was about 0.1 μm corresponding to 100 flagella $μm^{-2}$. Since the area of the flagellar field was about 4 $μm^2$, the total number of flagella can be estimated to be around 400. The flagella had a diameter of about 15 nm and so the volume fraction of flagella in the tuft was only about 2%; the remaining bulk of the tuft consists only of water surrounding the flagella. In the living bacterium, the structure of the flagellar tuft is maintained through hydrodynamic coupling between the individual flagella. However, the natural shape of the tuft remained intact in cells fixed with OsO_4 or OsO_4-containing fixatives.

The Sudan black staining inclusions dissolved during preparation for TEM and left only empty spaces (Fig. 3(a)). But the TEM photographs also revealed another type of smaller, very electron dense inclusions of unknown nature (Fig. 4(d)).

3.2. Swimming behaviour

Living "Ovobacter" cells always swam and were never observed to attach. It was obvious that bright white light from the microscope lamp acted as a deterrent and so the cells quickly disappeared from the field of view. We therefore used a red light filter for all recordings of swimming cells since the bacteria were unaffected by red light. One of the striking properties of "Ovobacter" was its high swimming velocity; most cells swam at a velocity of 0.6–0.7 mm s^{-1}, and some cells swam up to 1 mm s^{-1} (Fig. 5). High-speed video recordings at high magnification revealed that swimming was accomplished by a rapid counter clockwise rotation – if viewed from the rear – of the backwards-bent flagellar tuft as shown schematically in Fig. 6; it functioned similar to a propeller. The angular momentum of the flagellar motor caused a clockwise rotation of the cell. The hydrodynamic torque also contributed to the rotation of the cell and due to the asymmetrical structure of the cell, the resulting rotational axis was not aligned with the velocity vector. The resulting swimming path was a right-handed helix (clockwise when viewed from behind) with an axis that was parallel to the rotation axis of the cell (Fig. 7). The flagellar tuft always pointed against the axis of the helical swimming path. The radius of the helical swimming path is 2–3 μm, the pitch 5–10 μm and the period 10–20 ms. Rotation rates of the cells were therefore within the range of 50–100 Hz. Since the flagellar tuft made one turn for each turn of the cell, the rotation of the tuft relative to the cell must be 100–200 Hz. The swimming velocity of the cell was proportional to the angular velocity of the flagellar tuft.

Cells could abruptly change swimming direction. This was not a classical tumbling in which flagellar movement comes to a temporary halt or reversal, since there was no deceleration followed by acceleration during turns. Turning angles were typically around 180° and almost always within the range of 110° and 270° (Figs. 5 and 8). We could not determine exactly how the cells manage to turn around in terms of changes in flagellar motion pattern.
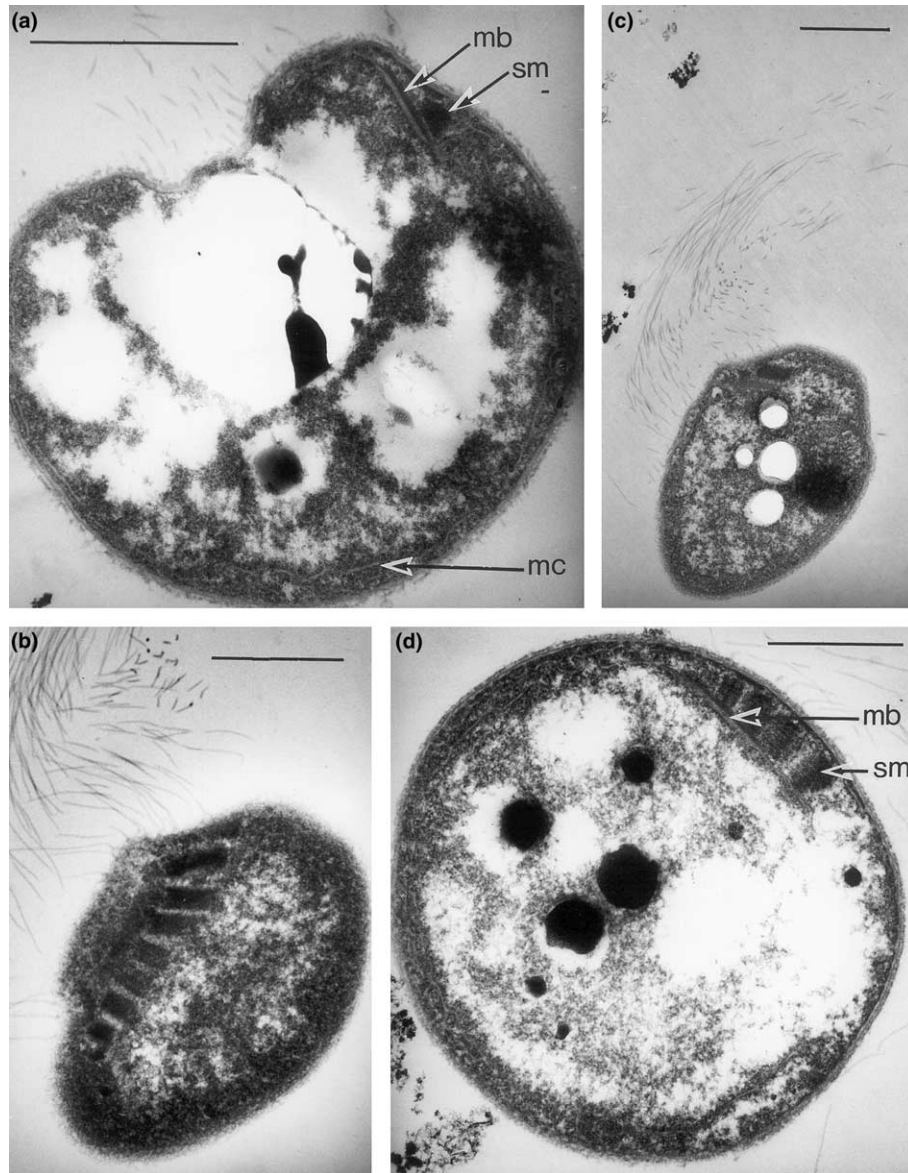
Fig. 3. TEM sections of Ovobacter propellens. (a) Transversal section (ventral side to the right; anterior end with flagellar groove at the top); (b) section close to the right side; (c) section through the "ventral side"; (d) oblique section through the central part of the cell. sm: stacked membranes; mb: membrane band; mc: membrane channels. All scale bars: 1 μm.

3.3. Behaviour in O_2-gradients

The behaviour of "Ovobacter" in an oxygen gradient is shown in Fig. 8. Within the bacterial band the cells moved in random directions. When they approached O_2-tensions of about 0 or about 1% atmospheric saturation they would turn around and almost always manage to return to the narrow interval of O_2-tensions between 0 and 1% atm. sat. Fig. 9 shows the distribution of the cells within the band that was typically about 150 μm wide. The O_2-gradient and the frequency of turning are also shown. The turning rates were sufficient to send almost all cells back to the band if they had ventured outside. However, cells that had arrived in the com-

pletely anoxic region did no longer turn, perhaps because there was then no longer any cues in terms of changing O_2-tension. Turning rates could not be reliably estimated for cells moving above an O_2-tension of about 1% atm. sat. due to the low number of observed cells.

4. Discussion

The phylogenetic position and the type of energy metabolism are two essential questions that we cannot answer. This would require isolation or at least some sort of selective enrichment procedures that could provide cultures with few contaminants. It is likely that
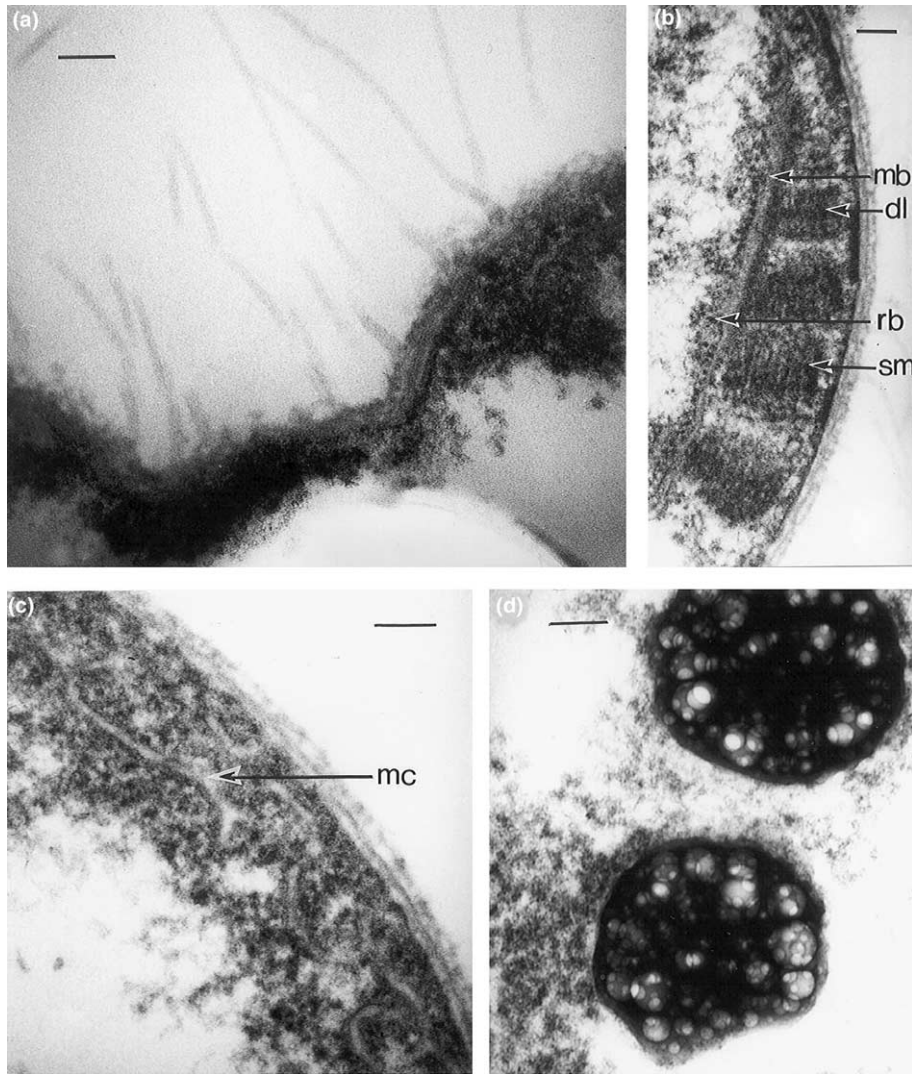
Fig. 4. TEM sections of Ovobacter propellens. (a) Section though flagellar groove ("ventral" side to the right); (b) section through the membrane band and stacked membranes. (c) cell wall and internal membrane channels; (d) electron dense inclusions. sm: stacked membranes, mb: membranelle band; mc: membrane channels; dl: dark layer; rb: ribosomes. All scale bars: 0.1 μm.
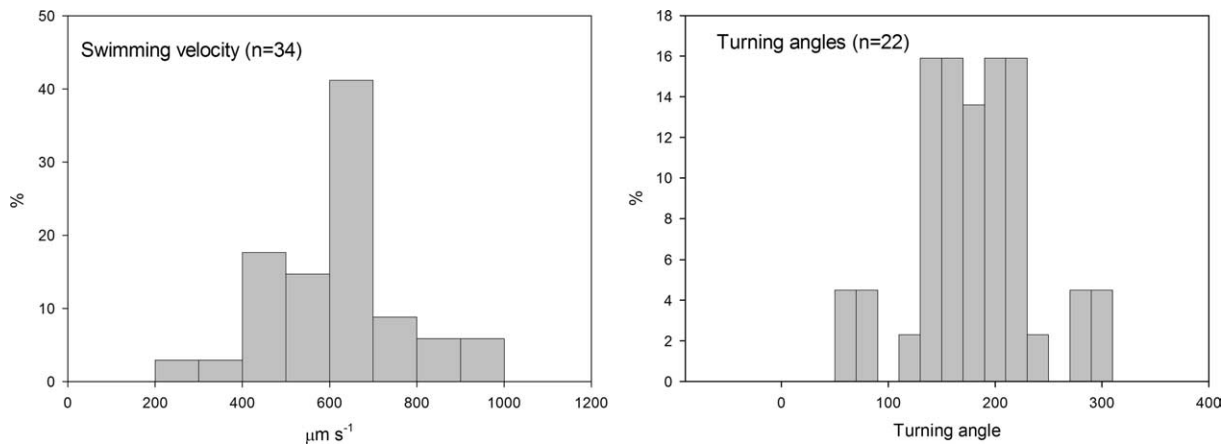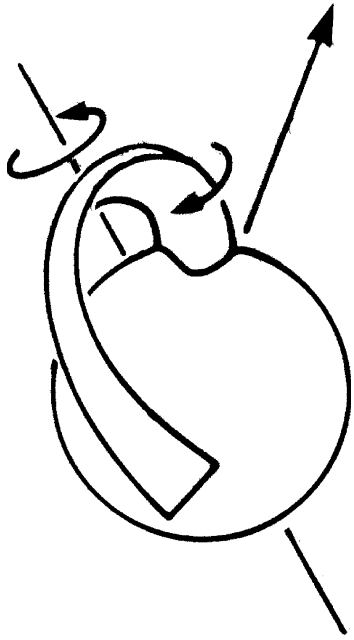

Fig. 5. Swimming velocities (left) and turning angles.

Fig. 6. Schematic presentation of swimming in Ovobacter. The flagellar tuft rotates counter clockwise and makes the cell rotate clockwise if viewed from the back. Due to asymmetries the velocity vector is not aligned with the rotational axis. The velocity vector therefore rotates around the rotational axis leading to a helical swimming path.
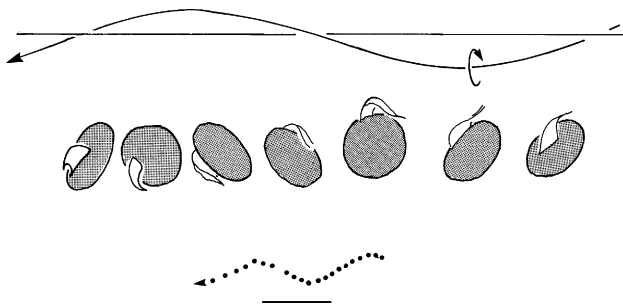

Fig. 7. Above: positions taken of an Ovobacter cell as it swims along its helical path. Below: high-speed video recording of a swimming Ovobacter cell; the time interval between dots is 1.5 ms and the scale bar is 5 μm.

Fig. 8. Swimming tracks of Ovobacter in an O_2-gradient. The scale bar is 50 μm and the time interval between the dots is 26 ms. Dashed lines indicate approximate positions of O_2-tension isopleths with 0% and 1% atm. sat. Due to the low time resolution in this presentation, the helical path is not evident. It is seen that the cells turn back when they encounter anoxia or O_2-tensions above 1% atm. sat.

"Ovobacter propellens" makes a living through the oxidation of some sort of reduced compounds diffusing from the anaerobic zone. The cells do not deposit sulphur granules or ferric hydroxide, making reduced sulphur compounds or ferrous iron unlikely substrates, although they cannot be totally ruled out. Other possibilities would be as oxidisers of CH_4, NH_4^+, H_2, or organic compounds such as acetate. Its preference for very low O_2-tensions is striking since most microaerophilic bacteria appear to prefer O_2-tensions around 4% atm. sat. Since it has recently been shown that some bacteria with an anaerobic metabolism actually show attraction to microaerobic conditions (e.g. [10]), it is finally a possibility that "Ovobacter" has some type of anaerobic energy metabolism.
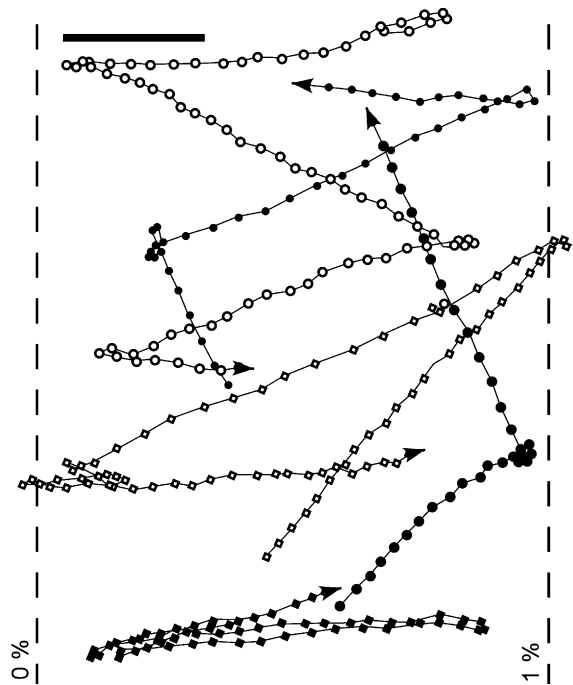
The studied organism has – as far as structure is concerned – many unique traits. These include the unusual asymmetric structure of the cell, the huge number of flagella, and the peculiar internal membrane structures. In spite of the many unknowns about the nature of this creature, it is very characteristic and the living cells can unambiguously be identified under the light microscope even at low magnifications. We have not yet attempted a phylogenetic analysis because we were not able to isolate pure samples among the rich diversity of other bacteria. Nevertheless, we find it justifiable to provide the bacterium with a name, and we have chosen one that refers to its shape and to its swimming mechanism.

The functional significance of the membrane structures along and beneath the flagellar field is also unknown. They might serve as mechanical enforcement of the flagellar groove, as sites for ATP-generation that again could provide the proton-motive force for driving the many flagella, or as an isolation between two groups of flagella (inside and outside the groove) that could work independently during turning – but these suggestions are all speculation.

A helical swimming path is also found in other large bacteria such as *Thiovulum* [2] and in various protists
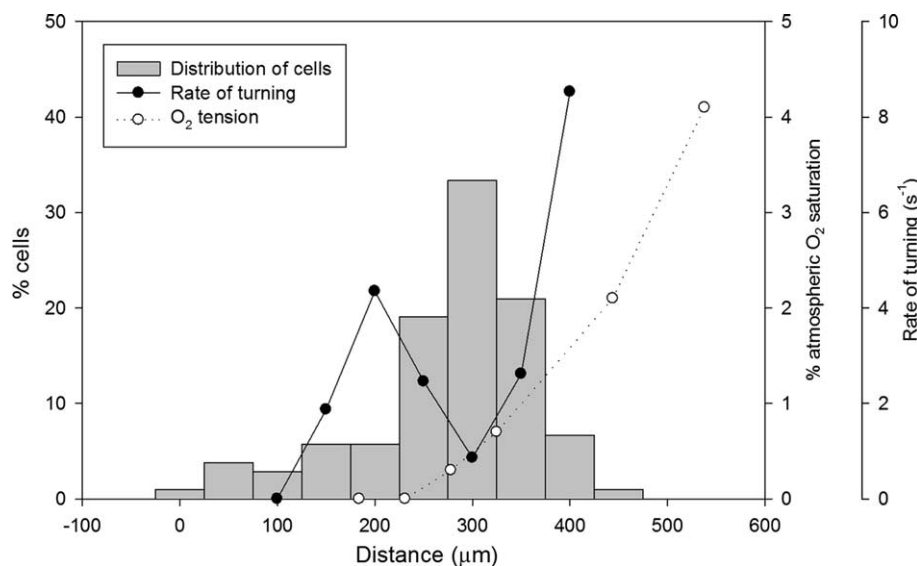
Fig. 9. Distribution of Ovobacter cells in an oxygen gradient. The frequency of turning (per cell per second) is also shown.

(e.g. [11]). A helix simply represents a default swimming pattern of small organisms [12] although in smaller bacteria the rotational component of Brownian motion distorts this pattern [13]. Many ''gradient'' bacteria that live in steep O_2-gradients swim fast. This may be considered as an adaptation to vertically migrating oxyclines. Typically, swimming speeds for such organisms are 2–300 μm s^{-1} [1] or up to 600 μm s^{-1} in the case of *Thiovulum* [2], but ''Ovobacter'' approaches 1 mm s^{-1} and is therefore probably the fastest bacterium recorded so far. It is likely that ''Ovobacter'' applies the kinematics of helical swimming for turning. This implies that the cells temporarily changes a component of the rotational velocity, thus turning the axis of the cell's rotation [12,14]. Such a mechanism for steering has been demonstrated in *Thiovulum* [2] and in many protists (e.g. [11]). But we were unable to observe exactly what happens when ''Ovobacter'' cells turn their direction of swimming.

It is generally assumed that for bacteria (and for most eukaryotic microbes) body sizes and swimming speeds are so small that swimming do not significantly enhance uptake of dissolved low-molecular-weight substrates: at the scale of microbes transport by diffusion is much faster than transport by advection. However, in the case of ''Ovobacter'', with its relatively large cell size and fast swimming, such an effect cannot be ruled out a priori. A crude feeling for the magnitude of the ratio between advective and diffusional transport is given by the dimensionless Péclet number (also named Sherwood number): $Pe = L \times v/D$, where L is length, v is swimming velocity and D is the diffusion coefficient [15]. If $Pe > 1$ then advective transport may be significant. The value of D is about 2×10^{-5} cm^2 s^{-1} for dissolved O_2 and if we assume a cell length of 4×10^{-4} cm and a swimming velocity of 0.07 cm s^{-1}, we have $Pe = 1.4$ and so some importance in terms of uptake of solutes cannot be ruled out. More exact numerical simulations indicate that ''Ovobacter'' can gain about 20% in terms of O_2 uptake by swimming relative to being stationary (R. Thar, unpublished results).

While the swimming velocity of ''Ovobacter'' is unusually high, the angular velocity of the flagellar motor is not unusual. It may, for example, exceed 100 Hz in *Escherichia coli* [16], even though this species swims 20 times slower than ''Ovobacter''. In ordinary bacterial flagella the efficiency is very low – only about 1% of the mechanical energy is converted to translation of the cells because the flagella slip as they translate through the water [17]. The underlying cause for the high speed of ''Ovobacter'' must be an increased energetic efficiency related to the huge flagellar tuft relative to single flagella or to slender tufts formed by only a few flagella.

The classical model for chemosensory behaviour in bacteria implies temporal gradient sensing in which the rate of change in concentration of a repellent or attractant affects the rate of tumbling. The new swimming direction following a tumble is typically uncorrelated with the previous swimming direction; that is, tumbling means a random reorientation in swimming direction. For species that must orient themselves in linear concentration gradients it is more efficient that changes in swimming direction are about 180° relative to the previous swimming direction whenever they encounter adverse conditions. In this respect, ''Ovobacter'' accords with many other bacteria that have specialised for life in O_2-gradients [1]. On the other hand, we found no evidence that the cells can orient themselves in gradients through helical klinotaxis [14] as has previously been demonstrated in *Thiovulum* and for an unnamed vibrioid species [5–7].

5. Description of "*Candidatus* Ovobacter propellens"

"Ovobacter propellens" (L. neutr. n. *ovum* egg; L. v. *propellere* to drive forth). Cells are ovoid in shape and measure 4–5 μm in diameter. The cytoplasm is concentrated along the periphery of the cells surrounding a central vacuole that contains Sudan black staining inclusions. On the anterior, somewhat flattened side of the cell an oblong groove is found along about 3/4 of the cell. It contains most of the flagella, but to one side flagella also occur outside, but parallel to the groove. There are altogether about 400 flagella per cell and they form a prominent tuft that bends backwards. Its counter clockwise rotation propels the cells and leads to a right handed, helical swimming path. Intracellular membrane channels are abundant along the periphery of the cell; they represent invaginations of the inner cell membrane. A membrane fold is also found along one margin of the groove; peripheral to this system a group of 12–13 stacked membranes are found at regular intervals along one side of the groove so that the cell structure shows a pronounced asymmetry. "Ovobacter propellens" cells always swim with an average velocity of 600–700 μm s^{-1} and some cells may attain a velocity of 1 mm s^{-1}. The cells avoid intense light. The species was found in the surface layers of sulphidic marine, shallow-water sediments along the west coast of the Sound, Denmark. It is microaerophilic and lives in steep O_2-gradients at O_2-tensions between 0% and 1% atm. sat.

Acknowledgements

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