Oxygen consumption of individual cable bacteria

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The electric wires of cable bacteria possibly support a unique respiration mode with a few oxygen-reducing cells flaring off electrons, while oxidation of the electron donor and the associated energy conservation and growth is allocated to other cells not exposed to oxygen. Cable bacteria are centimeter-long, multicellular, filamentous Desulfobulbaceae that transport electrons across oxic-anoxic interfaces in aquatic sediments. From observed distortions of the oxic-anoxic interface, we derived oxygen consumption rates of individual cable bacteria and found biomass-specific rates of unheard magnitude in biology. Tightly controlled behavior, possibly involving intercellular electrical signaling, was found to generally keep <10% of individual filaments exposed to oxygen. The results strengthen the hypothesis that cable bacteria indeed have evolved an exceptional way to take the full energetic advantages of aerobic respiration and let >90% of the cells metabolize in the convenient absence of oxidative stress.

INTRODUCTION

The rise of molecular oxygen O2 in the atmosphere during the Great Oxidation Event (2.4 to 2.0 billion years ago) posed a grand challenge to life (1). Some microorganisms evolved strategies to cope with the reactive oxygen species (ROS) superoxide, hydrogen peroxide, and hydroxyl radical that form during oxygen reduction and react with various cellular components causing detrimental effects and undermining cell viability (2–4). Other microorganisms, including the emerging eukaryotes, evolved to take advantage of oxygen as a very potent electron acceptor.

Today, anoxia still prevails in wet environments such as sediments, biofilm, guts, and feces because of the poor solubility and slow diffusion of oxygen in water (5). Over the course of evolution, microorganisms have developed different molecular and behavioral strategies to live at the oxic-anoxic interface, where they compete for electron donors released from anaerobic processes and jointly condense the activity to a very narrow zone with low oxygen concentrations (5, 6). A peculiar strategy circumventing the crowd at the interface is put in place by the filamentous cable bacteria. They exploit multicellularity and electric wires made of a yet unknown conductive material to spatially bridge anoxic and oxic environments with long-distance electron transport using sulfide as an electron donor (7, 8). By means of gliding motility with a speed of up to 2.2 μm s−1, cable bacteria have been observed to maintain their distinctive interface-spanning position with some segments curled around the oxic-anoxic interfaces and other segments connecting straight back to the sulfide source (9). With this partial exposure to oxygen, they do not align with the conventional thinking of organisms, metabolizing either with or without the presence of oxygen at any given time. Specifically, the unique allocation of the coupled half reactions of the basic redox processes to different cells raised the question of how energy conservation and subsequent biomass accrual are distributed among the cells. Genomic inspection found that cable bacteria do not have the conventional membrane-bound terminal cytochrome C oxidase linking oxygen reduction to energy conservation (10). Hence, it was proposed that they had optimized oxygen reduction for high speed in a few cells exposed to oxygen, while energy conservation and assimilation could be confined to the sulfide-oxidizing cells in the anoxic zone, free from the oxidative threat. A study of the succession of cable bacteria in marine sediment found that only 10% of the total cable bacteria biomass resided in the oxic zone of marine sediments (11). A conservative estimate based on the current density and the assumption that all cells in the oxic zone were equally active led to a cell-specific oxygen consumption rate 4 to 14 times higher than in other sulfide oxidizers of comparable cell size (11). Recently, it was observed that substantial biomass accrual was only seen in filament fragments retrieved from the anoxic zone after 19 to 24 hours of incubation with an amino acid tracer (10) or 15N and 13C tracers (12). Furthermore, cyclic voltammetry has showed substantial capacity to catalyze electrochemical oxygen reduction (12).

The aim of the present study was to use an individual-based approach to test whether cable bacteria indeed have evolved a strategy to “get the best and avoid the rest” of oxygen metabolism by means of electric currents, extremely compacted consumption, and controlled positioning across oxic-anoxic interfaces. We directly observed live, cable bacteria at oxic-anoxic interfaces in microchambers with a clonal enrichment culture and obtained individual and, in turn, cell-specific oxygen consumption rates from their visible distortions of oxygen gradients.

RESULTS AND DISCUSSION

Individual oxygen consumption rates obtained from visible distortions of the oxic-anoxic interface

Custom-made glass chambers (“trench slides”; Fig. 1A) were designed to let cable bacteria migrate from anoxic sediment out into an observation window to meet oxygen near the air-exposed edge. As pure cultures of cable bacteria are not available, the slides were inoculated with a sediment-based enrichment of Candidatus Electronema sp. GS, a freshwater species of cable bacteria with a medium thickness of 1 to 2 μm (13, 14, 10). Various kinds of swimming bacteria rapidly formed a distinct, visible veil 0.3 to 2.6 mm from the edge (Fig. 1). Linear oxygen concentration gradients from air saturation at the edge to zero in the veil, as visualized in two dimensions by planar optode–based chemical imaging (15, 16), documented that the veil bacteria were accurate markers of the oxic-anoxic interface and consumed all the intruding oxygen within a zone of 10 μm and at oxygen concentrations below 5 μM (Fig. 1, D and E). The linearity of the oxygen gradient also demonstrated that the transport
The individual variation makes sense, as electron flow in a cable bacterium depends on many factors, including length, success in synchronously reaching electron sources and oxygen, and the extent of damage to the internal electric wires (18, 19). To record the metabolic rate of individual bacteria in real time, and even in a natural gradient environment, is exceptional. To the best of our knowledge, only one previous study achieved this type of measurement by means of oxygen microsensors on a single, giant (diameter, 220 \( \mu m \)) Thiomargarita namibiensis cell (20).

The hitherto highest biomass-specific oxygen consumption rate of a prokaryote reported in literature belongs to Desulfovibrio termitidis. When incubated with hydrogen and 20 \( \mu M \) oxygen concentration, \( D. \) termitidis showed an oxygen consumption rate of 1570 nmol O\(_2\) mg protein\(^{-1}\) min\(^{-1}\) (2, 21). For cable bacteria, an even higher rate of 2200 nmol O\(_2\) mg protein\(^{-1}\) min\(^{-1}\) for the oxygen-exposed part was calculated using standard factors to convert from the individual rates and the biovolume measures (see Materials and Methods). \( D. \) termitidis has both high- and low-affinity cytochrome c oxidases (genome accession number: GCA_000504305.1), which are involved in the energy conservation using hydrogen as an electron donor. Cable bacteria, however, do not have any canonical terminal oxidases, including those present in Desulfobulbus propionicus from the same family (10, 13). In their absence, it has been proposed that a unique cytochrome-truncated hemoglobin fusion protein might be the catalyst for a condensed periplasmic oxygen reduction (13). The presence of conductive fibers in the periplasm (8, 22, 23) indirectly backs this hypothesis, as electrons would be unloaded in the periplasm onto the fusion protein, which has no apparent anchorage to the cytoplasmic membrane and therefore no means of energy conservation; in addition, oxygen would thus be quickly reduced in direct proximity of the fiber. However, the complete oxygen reduction pathway in cable bacteria is not obvious. Whether and how the truncated hemoglobins, the cytochrome moiety, or other enzymes contribute to the oxygen reduction to water requires elucidation.

**Cell-specific oxygen consumption**

By measuring the oxic part of the filament and assuming 3 \( \mu m \) as a conservative estimate of the cell length (13), the individual rate could be readily converted into an average per-cell oxygen consumption rate estimate (fig. S5A and table S1). Despite expectations, no statistically significant correlations between the cell-specific oxygen consumption rate estimates and the lengths of parts on either side of the oxic-anoxic interface were found (Fig. 2). However, the large difference of the mean and median rate, 500 and 200 fmol O\(_2\) cell\(^{-1}\) day\(^{-1}\), respectively, reflected a clustering with a large cluster below 250 fmol O\(_2\) cell\(^{-1}\) day\(^{-1}\) and a smaller cluster between 600 and 2140 fmol O\(_2\) cell\(^{-1}\) day\(^{-1}\) (fig. S5). It is tempting to suggest that the low-rate cluster represents cable bacteria in a situation where the electron flow was limited on the donor side, while the high rates indicate oxygen limitation of the current and hence represent maximum cell-specific capacity (fig. S5, A and B). In a recent cyclic voltammetry experiment,
an oxygen consumption rate of 6134 fmol O₂ cell⁻¹ day⁻¹ was estimated (12). The marine cable bacteria used in the latter had more than three times larger cell volumes (3- to 5-μm diameter, as opposed to the Candidatus (Ca.) Electronema cells used in this study with an average diameter of 1.42 μm). Taking into account the cell volume difference, the high rates between 600 and 2140 fmol O₂ cell⁻¹ day⁻¹ obtained in this study indicate that the rates obtained from cyclic voltammetry are indeed realized in live cable bacteria with natural, internal currents.

Behavior of cable bacteria at the oxic-anoxic interface

Cable bacteria that had positioned themselves across the oxic-anoxic interface in the trench slides only had 8.1 ± 6.4% of their bodies protruding into the oxic zone (fig. S5B), meaning that only few cells were responsible for the oxygen reduction of the whole cable bacterium. This is in line with previous measures of 10% of the cable bacteria biomass in the oxic zone of marine sediment (11). The ratio between the distances from the veil to the furthest oxic cell and to the edge, respectively, was used to calculate the maximum oxygen concentration that cable bacteria experienced, considering a linear oxygen gradient as confirmed by planar optode–based imaging. Longer cable bacteria, extending far away from the oxic zone toward the sediment and with a steady current, tended to keep cells below 14% air saturation (movie S1), indicating a phobic response to oxygen with a threshold a little higher than observed in another sulfide-oxidizing, gliding filamentous bacterium, Beggiatoa spp. [5% air saturation; (24, 25)]. One cable bacterium, observed for more than 12 hours, kept the same position across the oxic-anoxic interface, dynamically readjusting to small concentration changes (movie S2). Generally, cable bacteria inverted the direction of motion for short-term readjustments to the oxygen concentration every 60 s. Shorter cable bacteria that transiently dipped in the oxic zone without consuming oxygen showed similar short-term readjustments (movie S1), suggesting that the taxis is a response to oxygen rather than to changes in potential electron delivery as reflected in wire electric potentials. This aligns with the large cluster of observations of cells running far below the capacity as discussed above. Motility controlled so tightly by oxygen being experienced by only the very end of the filament raises questions on how distant segments are informed when to reverse gliding. In the filamentous cyanobacterium Phormidium uncinatum, lateral transmission of transmembrane potential ΔΨH₂, from a transiently illuminated segment of the trichome initiates motility at the other end of the trichome at least 2 mm away and in a time frame that rules out any role of signaling by diffusion of molecules (26–28). Similar rapid responses over millimeters distance with filamentous Beggiatoa spp. also calls for electrical rather than chemical signaling (24). Cable bacteria may instead use global changes in the potential of the internal wires as a second type of electrical signaling coordinating the behavior of a multicellular, filamentous bacterium (14, 29). By keeping less than a tenth of the filament in the oxic zone (table S1) (11), the few oxic cells must flare off a considerable electron load to oxygen, which should result in the production of ROS. The high expression of proteins such as catalases, superoxide reductase, rubrerythrin, and GroEL/ES chaperonins in a previous study suggest that cable bacteria are indeed exposed to substantial oxidative stress (10). ROS have detrimental effects on viability, growth, and cell motility, as many enzymes responsible for the anabolism are inhibited (2, 3, 6). Despite expression of superoxide reductase and rubrerythrin that can dispose of ROS (10), apparently irreversible structural changes of oxygen-exposed cells were observed during long-term observations (movie S2). Even if these cells were dying, this loss might, from an evolutionary point of view, still be worth the price (10, 12), as >90% of the filament lies in an oxygen-free environment. This strategy allows cable bacteria to harvest the power of a superior electron acceptor such as oxygen while, at the same time, circumventing the negative consequences that come with its reduction.

This study confirms that cable bacteria’s ingenious adaptation to life at oxic-anoxic interfaces encompasses electricity, putatively in multiple ways, to communicate among distant cells and assign the troublesome reduction of oxygen to a few cells with possibly the most intense oxygen consumption in biology. More live observations of individual cable bacteria may also help to understand other essential features of this unique form of life, such as management of energy storages, allocation of growth, and competition among cable bacteria.

MATERIALS AND METHODS

Study design

Cable bacteria were observed to generate distinct distortions of the oxic-anoxic interface in gradient systems, and we realized that this allowed measurements of the rate and location of oxygen consumption in individual bacteria. This novel approach was used in the present study to directly test the hypothesis that cable bacteria can limit oxygen reduction to a few cells with high consumption rates (10, 12). Data from independent individuals of a single-strain population established in seminatural gradient systems were collected until robust measures of the variability and biomass-specific capacity of oxygen consumption were obtained. The new method also appeared to enable observations over a time span of days, and from video recordings, additional data and observations concerning the behavior of cable bacteria were extracted.

Preparation of cable bacteria for observation

Slides for microscope observation of cable bacteria positioning at the oxic-anoxic interface were prepared as described in (9, 14). Specifically, four glass strips were glued together on a microscope slide, to form a 25 mm by 60 mm frame with a central “trench” of 25 mm by
10 mm by 1 mm. The trench was filled with ca. 0.25 ml of sediment enriched with the freshwater cable bacterium *Ca. Electronema* sp. GS described in (10), and the trench and frame was covered with a coverslip while flushed with N₂-sparged Milli-Q water to avoid entrapped air. Cable bacteria filaments would then move out between the coverslip and the frame and toward the edge, where oxygen diffused in from the air. The slides were kept in 50-ml Falcon tubes containing a piece of moist paper to avoid drying, and 1 hour before visualization, they were retrieved and wrapped into vapor-tight, oxygen-permeable transparent plastic foil, which had no notable impact on oxygen penetration (Vita, MH Line) (fig. S2).

**Microscopy and imaging**

Images used for the calculation of oxygen consumption rates were acquired within 48 hours from the preparation of the slide with 10× and 40× objectives and phase contrast using a Zeiss Observer Z1 (Zeiss, Göttingen, Germany) inverted microscope equipped with a PALM-automated stage and an AxioCam ICC1 (Zeiss, Göttingen, Germany) color camera. Trench slides were prepared the same way throughout the experiment; at times, more than one distortion within the same slide were observed. In these instances, the spot where the image was taken was marked to avoid coming back to the same cable bacterium/duplication. The microscope scaling and length measurement were calculated using calibrations of the microscope software (Zen Blue Edition; Zeiss, Göttingen, Germany). Large micrographs spanning from the edge to the central trench were acquired using the automated tiles or panorama options of the software. The distance between the coverslip and the glass chamber (Z) was obtained by focusing first on the top of the coverslip and then on the top of the glass chamber’s upper surface; the thickness of the coverslip was then subtracted.

**Planar optode imaging**

The planar optode was obtained by dissolving 1.5 mg of platinum (II)‒5,10,15,20-tetrakis-(2,3,4,5,6-pentafuorophenyl)-porphyrin (Frontier Scientific, Utah, USA), 1.5 mg of Macrolex fluorescence yellow (Lanxess, Cologne, Germany), and 1 g of polystyrene (Merck) in 10 g of toluene (Sigma-Aldrich). The solution was spin-coated over 24 mm by 60 mm coverslips with a spin coater at 3000 rpm for 30 s (model WS-650MZ-23NPPB; Laurell, North Wales, USA) (13). Glass chambers for the visualization of the oxygen gradient were prepared as described above. The coverslip was laid down with the planar optode in contact with the sediment and the water phase. The images were acquired with a Zeiss Observer Z1 inverted microscope equipped with a pco.flim camera (pcO., Kelheim, Germany), controlled through the Nis Element (Nikon, Tokyo, Japan) and a LedHUB High-Power LED Light Engine controlled by the Omicron software (Omicron-Laserage GmbH, Rodgau-Dudenhofen, Germany). A blue (460 nm) light-emitting diode (LED) was used for excitation, and light was recorded in the red end of the spectrum. The LED was modulated at a frequency of 5 kHz, and the luminescence decay time, τ, was obtained as shown elsewhere (30). After calibration of the optode to known O₂ concentrations, the determined luminescence decay time was converted to the actual O₂ concentration using the simplified two-site model (30) (Fig. 1 and fig. S1). Calibration was performed by fixing a small tube (volume of around 2 to 3 ml) on top of the optode with ultraviolet glue (Loctite 3949, Henkel, USA) and filling it with water that could be gently bubbled with air/N₂ mixtures that were controlled by a home-built gas mixer using battery-powered digital mass flow meters (Vögtlin Instruments GmbH, Switzerland). Image processing was performed with ImageJ (https://imagej.nih.gov/ij/) using a self-written macro to convert the imaged lifetime images into O₂ images.

**Calculation of oxygen consumption of individual cable bacteria**

Distortion of the oxic–anoxic interface at intersect with individual cable bacteria (*n* = 24) was used to calculate the bacterium’s oxygen consumption rate, *R*, by means of Fick’s first law of diffusion

\[
R = D \times C_0 \times Z \times \int_{x=0}^{x=m} \left( \frac{1}{Y(x)} - \frac{1}{Y_b} \right) dx
\]

using the oxygen diffusion coefficient *D* = 2.1 × 10⁻⁵ cm² s⁻¹; air saturation oxygen concentration, *C₀* = 284 μM at 20°C; chamber height, *Z*; and the distances to the edge from the base line veil, *Y_*b, and from the veil with distortion, *Y(x)_b*, along the inspected length of veil, *x*.

In practice, the distances were obtained for 10 equally spaced locations along the observed length of veil including the distortion and the base (fig. S3). When more cables were sticking through the veil, the overall length of cable bacteria in the oxic zone was considered; when calculating the % fraction of the filament in contact with oxygen, single cable bacteria’ length was considered (table S1). Sizeable sediment particles and ciliates in the veil’s proximity were occasionally observed to cause distortions, and those regions were not considered for cable bacterium observations. One outlier was excluded from the calculation of the mean and median, but it was nevertheless reported (fig. S5 and table S1).

The individual oxygen consumption rates were compared with *D. termitidis* [reported in pmol O₂ mg protein⁻¹ mg⁻¹; (21)] by calculating the volume from the length of oxygen-exposed filament and the diameter, 1.7 μm, and converting it to protein content based on the conversion factors adopted by Makarieva *et al.* (31) (dry-to-wet weight ratio, 0.2; protein-to-dry weight ratio, 0.5). Cable bacteria do often contain inclusion bodies (10, 12). The presence of inclusion bodies (e.g., polyphosphate and polyglucose) would decrease the protein-to-dry weight ratio, and the protein-specific oxygen consumption rate would exceed even more that of *D. termitidis*. The individual oxygen consumption rate of a cable bacterium was also converted to a per-cell rate, considering the part in the oxic zone, and a conservative cell length of 3 μm (13).

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/7/eabe1870/DC1

View/request a protocol for this paper from Bio-protocol.

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