Single cell view on the assimilation of carbon and nitrogen by cable bacteria

Nicole M.J. Geerlings, Cheryl Karman, Lubos Polerecky, Stanislav Trashin, Karel A. As, Michiel V. M. Kienhuis, Silvia Hidalgo-Martinez, Diana Vasquez-Cardenas, Johann L. Post, Henricus T.S. Boschker, Karolien De Wael, Jack J. Middelburg, Filip J. R. Meysman

Contact: N.M.J.Geerlings@uu.nl

OBJECTIVES

To assess how the electron flow within single filaments of multicellular cable bacteria is coupled to energy conservation and biomass synthesis.

INTRODUCTION

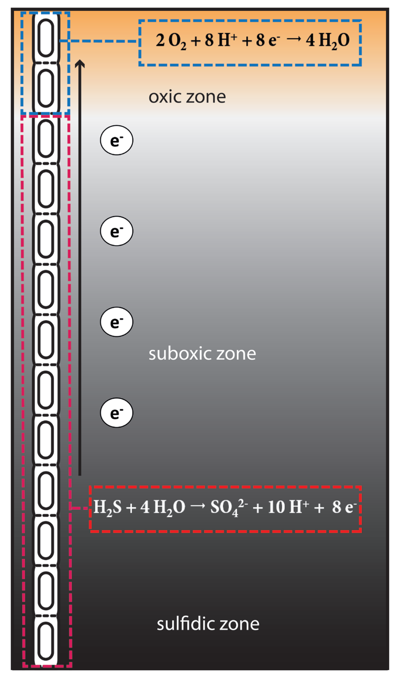

Figure 1: Conceptual image of a cable bacteria performing electrogenic sulfide oxidation within the sediment.

Cable bacteria are multicellular filamentous bacteria that stretch from the top of sediments where oxygen is present to lower anoxic regions where hydrogen sulfide is available. They gain energy by performing so-called "electrogenic" sulfide oxidation. The electrons generated by cells performing the anodic oxidation of sulfide are transported along the filament like a wire. These electrons are subsequently used for cathodic oxygen reduction by cells in the oxic zone[1] (Fig. 1).

This energetic division of labour allows cable bacteria to utilize resources that are widely separated in location[2]. This electrical connection between cells of the same filamentous organism raises questions about how the electron flow is coupled to energy conservation and biosynthesis.

RESULTS

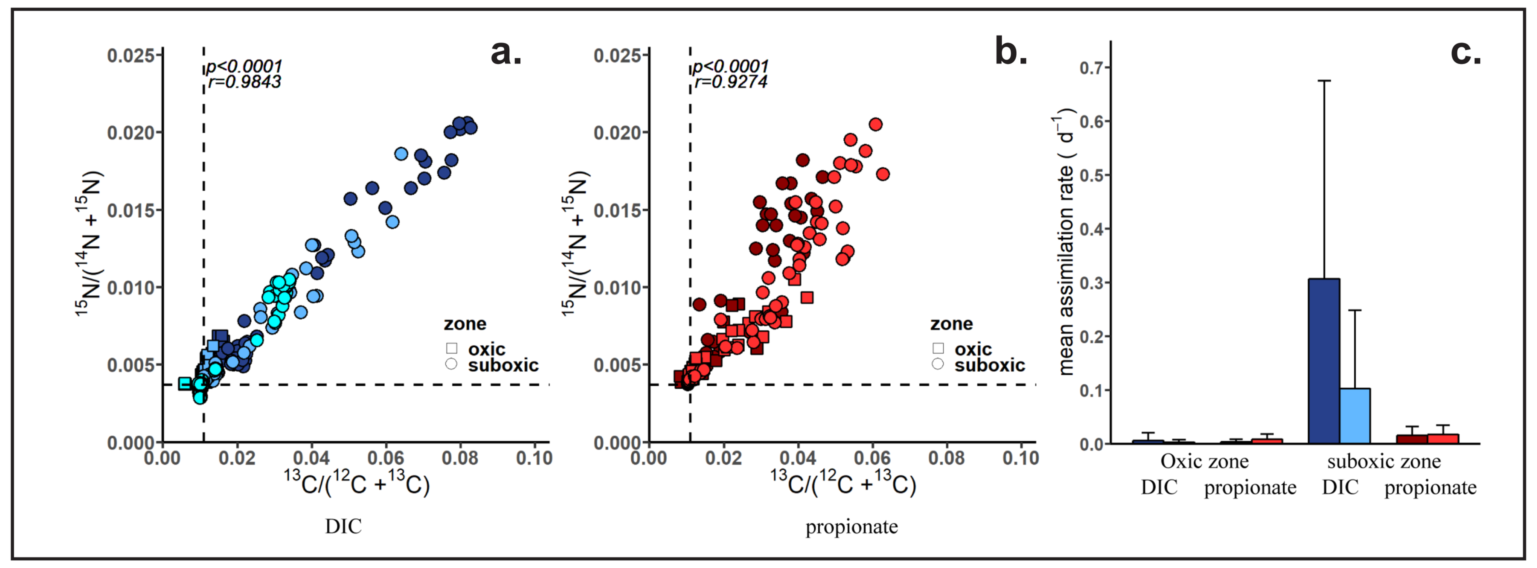

Figure 2: Mean ^{13}C and ^{15}N assimilation in individual cable bacterium filaments as measured by nanscale secondary ion mass spectroscopy (nanoSIMS). (a) Incubations with ^{13}C-bicarbonate and ^{15}N-ammonia. **(b)** Incubations with ^{13}C-propionate and ^{15}N-ammonia. Each data point represents the mean ^{13}C and ^{15}N uptake for an individual filament (n=524). Dotted lines represent the natural ^{13}C ratio (0.011) and the natural ^{15}N ratio (0.0037) for comparison. **(c)** Calculated mean assimilation rate for all filaments, differentiated between carbon source and redox zones.

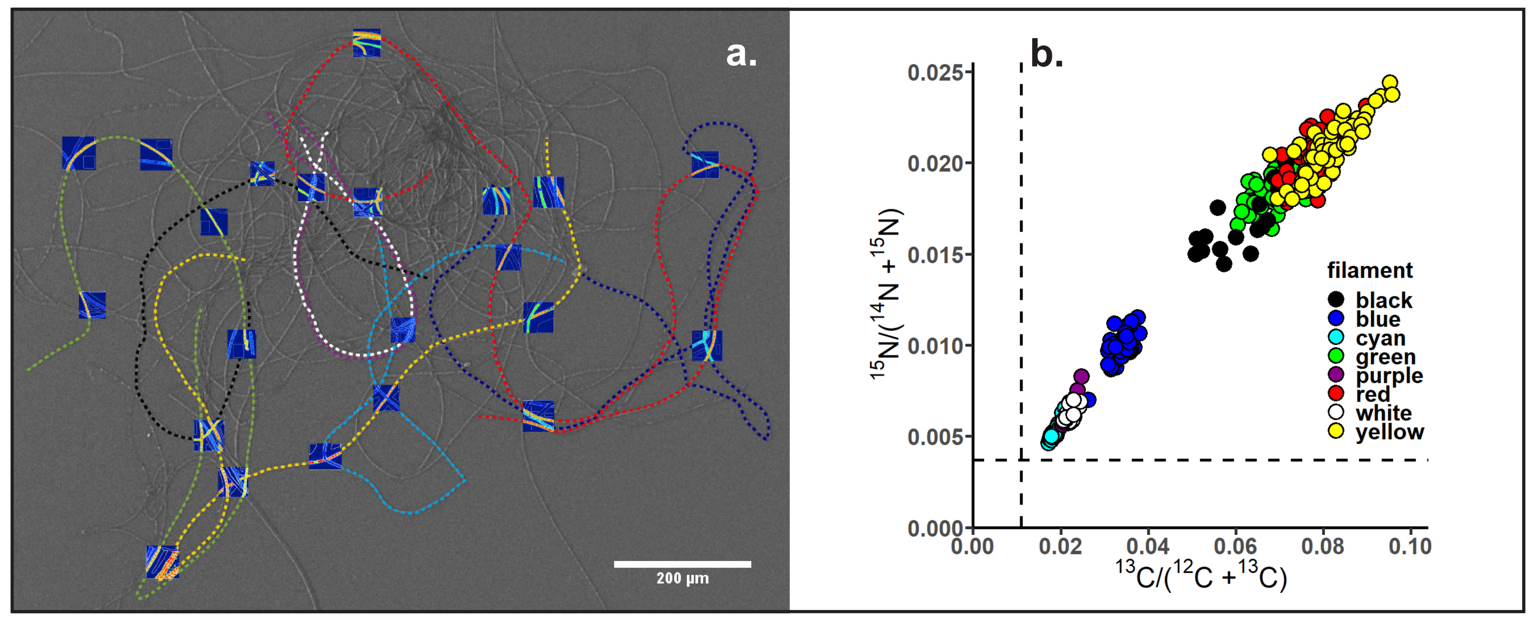

Figure 3: Variation in ^{13}C and ^{15}N assimilation of cells within individual cable bacterium filaments. (a) SEM image of a bundle of cable bacterium filaments from the suboxic zone from the incubation with ^{13}C-bicarbonate. Eight filaments were analyzed in detail by NanoSIMS – the investigated section of the filament is indicated by different colors. Multiple Regions of Interest (ROI) were imaged along the filament sections, and ^{13}C and ^{15}N uptake was determined for all cells within each ROI. NanoSIMS images of ^{13}C uptake are superimposed onto the SEM image. Scale bar is 200 µm. **(b)** Single cell ^{13}C and ^{15}N uptake for the filaments analyzed. Each point represents the averaged ^{13}C and ^{15}N uptake of a cell within a filament. Colors denote different filaments.

DISCUSSION

Cable bacteria mainly use inorganic carbon as a carbon source, there might be additional carbon fixation via propionate uptake. However, propionate uptake rates were only 5-9% of the bicarbonate uptake rates. Carbon and nitrogen assimilation in cable bacteria appears to be strongly coupled. This coupling is observed in numerous filaments from different sediment cores (Fig. 2).

Our isotope labeling data reveal a striking dependence of label uptake on redox zonation. Cells from the suboxic zone show high carbon assimilation, whereas cells in the oxic zone show little or no uptake of ^{13}C and ^{15}N. Biomass synthesis appears to be completely uncoupled from oxygen utilization (Fig 2).

^{13}C and ^{15}N labeling showed substantial variation of uptake between filaments (coefficient of variation between 46-70%), a detailed examination of eight active filaments (Fig. 3) revealed limited variability in label uptake within filaments (coefficient of variation between 5−16%) implying that there is some form of communication between cells within the same filament.

CONCLUSIONS

- Cable bacteria are facultative autotrophs.
- The uptake of C and N in the suboxic zone is remarkable homogeneous in cells belonging to the same filament suggesting some form of communication.
- Cells in the oxic zone have no capacity for biomass formation and energy generation. These oxic cells are likely optimized to get rid of electrons as soon as possible and can therefore be considered altruistic.

METHODS

1. Incubation of an enrichment culture

2. Labeling with $H^{13}CO_3^-$/^{13}C-propionate and $^{15}NH_4^+$ for 24h

3. Collecting individual filaments from the oxic and suboxic zone

4. Clean filaments with UHQ and dry on a gold-coated polycarbonate filter

5. Observe filaments with scanning electron microscopy (SEM)

6. Analyze isotope enrichment using nanoscale secondary ion spectroscopy

REFERENCES

[1] Pfeffer, C., Larsen, S., Song, J., Dong, M., Besenbacher, F., Meyer, R. L., et al. (2012b). Filamentous bacteria transport electrons over centimetre distances - Supplementary Information. Nature 491, 218–221. doi:10.1038/nature11586.

[2] Meysman, F. J. R. (2018). Cable Bacteria Take a New Breath Using Long-Distance Electricity. Trends Microbiol., 1–12. doi:10.1016/j.tim.2017.10.011.

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