Bacterial Phylogeny in vivo: cytochrome-c based Resonance Raman microscopy

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Cytochromes c (cyt-c) are ubiquitous heme proteins, essentially functioning as electron transfer proteins. They are found in the majority of life forms on Earth, with few exceptions among archaean extremophiles. The heme moiety of cyt-c, a porphyrin-structure embedded in the protein moiety (see Fig. 1), is a firm evolutionary constant; any change to this part of the molecule results in severe perturbation of the protein function. However, since bacteria often form multiple, specialized cyt-c dedicated to specific tasks involving electron transport, evolutionary change is abundant in the protein moiety of bacterial cyt-c.

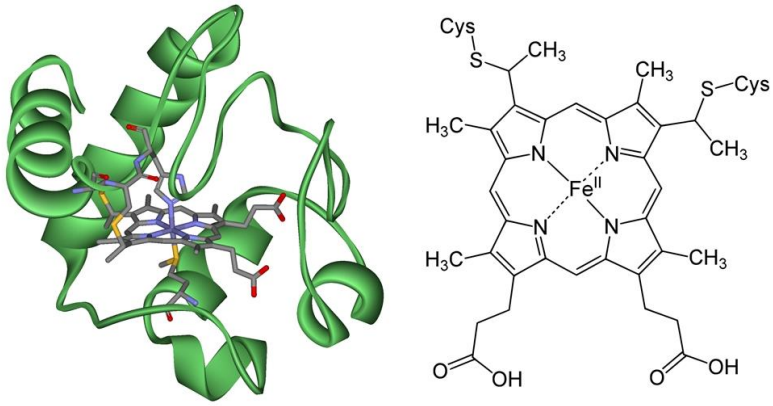

Figure 1 Schematic of cytochrome-c with the heme embedded in the protein moiety (left) and the basic structure of heme-c (right).

Confocal Resonance Raman Microscopy tuned for the porphyrin lattice vibrations of the heme-c is used to record the cyt-c Raman spectrum of individual bacteria cells in vivo and in situ. Hierarchical Cluster Analysis (HCA) then categorizes the Raman spectra into clusters based on their spectral similarity, thus determining the relative similarity of the cells' cyt-c content and therefore, how evolutionary close the analyzed bacteria cells are to each other.

In a proof of concept we determined the relative relatedness (phylogenetic distance) of three strains of native *Nitrosomonas* bacteria (*N. communis* Nm-02, *N. europaea* Nm-50, *N. eutropha* Nm-53) and the carotenoids-free mutant *Rhodobacter sphaeroides* DSM 2340^T based on the similarity of their cyt-c resonant Raman spectra recorded in vivo from individual cells kept in several planktonic cultures on different days. Spectra recorded from different cells of the same strain had a spectral similarity of over 95% independent of strain, sample, or sampled culture. The spectra of Nm-50

and its evolutionary close relative Nm-53 showed an overall spectral similarity of 84% to each other, whereas the spectral similarity dropped to 77% when either of them was compared to the more distant relative Nm-02. A comparison between *Rhodobacter* and the tested *Nitrosomonas* strains returned a spectral similarity or mere 46%. As can be seen in Fig. 2, the cyt-c resonant Raman analysis returns the same qualitative species relations as established by the standard procedures, although further work is required to determine correlation factors, the valid phylogenetic range, and the methods applicability to complex environmental samples [1].

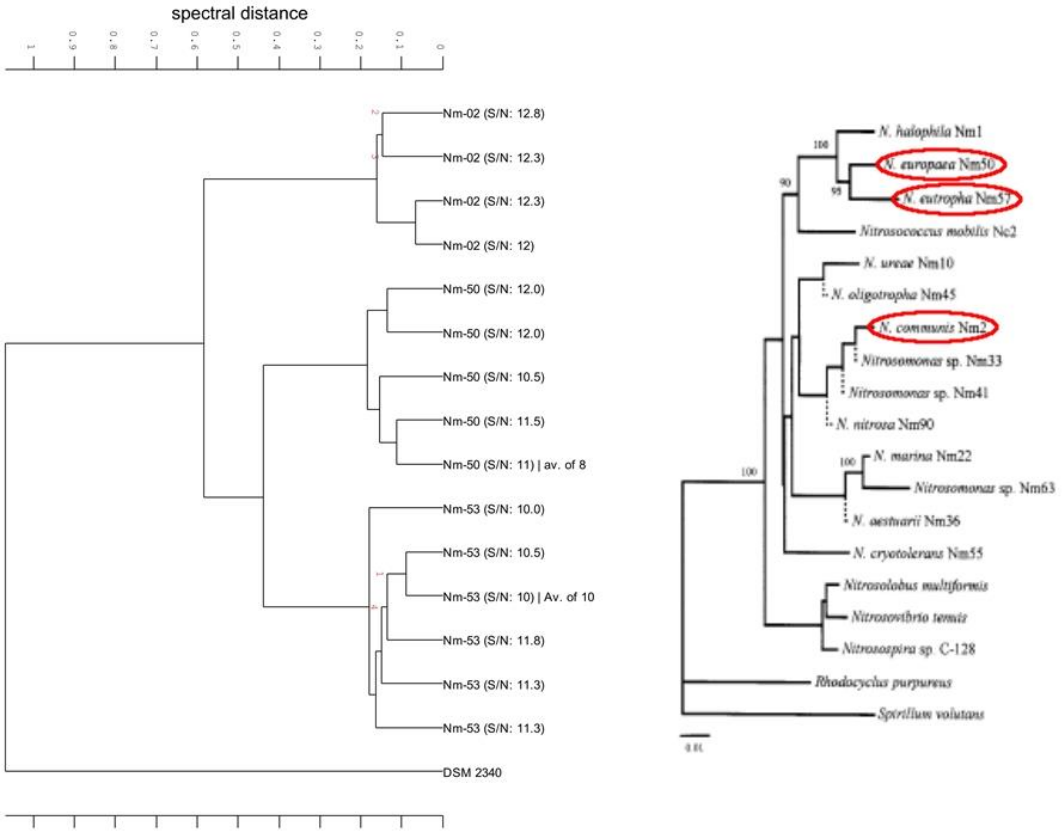

Figure 2 (left) HCA of cyt-c resonant Raman spectra recorded from individual cells of Nm-02, Nm-50, Nm-53, DSM 2340^{T}. Spectra were cut and vector-normalized to 600 – 1800 rel. cm^{-1} prior HCA. HCA-Algorithm: Weighted-Average-Linkage. S/N-range: 10 – 13. Software: OPUS 5.5 by Bruker. (right) Phylogenetic distance tree based on 16S rDNA sequences of the genus *Nitrosomonas* [2]. The circled species are clustered on the left.

ACKNOWLEDGMENTS

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REFERENCES

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