MICROBIAL ABUNDANCES AND METABOLIC FUNCTIONS OF SPONGE MICROBIOMES

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Abstract
Marine sponges are known to harbor dense and diverse microbial communities. In spite of considerable insights into the microbial diversity of marine sponges, quantitative information on microbial abundances and community composition as well as the functional gene repertoire remain scarce. Here we present our recent research contributions to the specific quantification of sponge microbes and to the elucidation of their possible functions.

Keywords: Porifera, Symbiosis, Microbiota, Mediterranean Sea, Red Sea

To gain quantitative numbers of sponge associated microorganisms, we established qPCR assays for the specific quantification of four bacterial phyla of representative sponge symbionts as well as the kingdoms *Eubacteria* and *Archaea*. We showed that the 16S rRNA gene numbers of *Archaea*, *Chloroflexi*, and the candidate phylum *Poribacteria* were 4-6 orders of magnitude higher in HMA (high-microbial-abundance) than in LMA (low-microbial-abundance) sponges. The actinobacterial 16S rRNA gene numbers were 1-2 orders higher in HMA over LMA sponges, while those for *Cyanobacteria* were stable between HMA and LMA sponges. Fluorescence *in-situ* hybridization (FISH) of *A. aerophoba* tissue sections confirmed the numerical dominance of *Chloroflexi*, which was followed by *Poribacteria.* Archaeal and actinobacterial cells were detected in much lower numbers (Fig. 1). By the use of fluorescence activated cell sorting (FACS) and whole genome amplification (WGA) as a primer- and probe-independent approach, the dominance of *Chloroflexi*, *Proteobacteria*, and Poribacteria in *A. aerophoba* was confirmed [1].

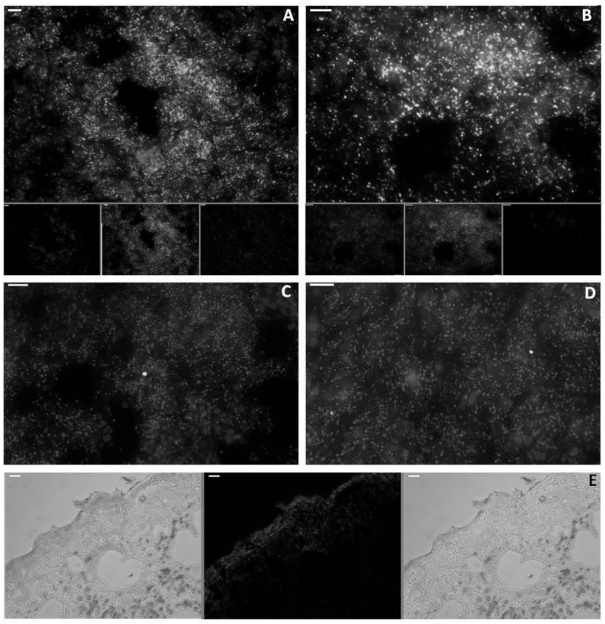

Fig. 1. Fluorescence in-situ hybridization on *A. aerophoba* sections for visualization. (A) *Poribacteria* using probe POR1130 (red), EUB338 probe mix (green) and DAPI staining (blue); (B) *Chloroflexi* with probe GNS934 (red); EUB338 probe mix (green) and DAPI (blue); (C) *Archaea* using probe Cren537 (green) and (D) *Actinobacteria* with probe HGC 237 (green), EUB338 probe mix (red) and DAPI; (E) light microscopy and cyanobacterial auto fluorescence in *A. aerophoba*. All red probes were cy3-labeled and the green probes were fluorescein-labeled. Additionally, DAPI was used for DNA-staining. Scale bars: 10µm (A-D), 50µm (E).

Secondly, the GeoChip 4 functional gene array was employed to interrogate the microbial functional gene repertoire of sponges (HMA and LMA) and seawater collected from the Red Sea and the Mediterranean. Altogether 20,273 probes encoding for 627 functional genes and representing 16 gene categories were positively identified. Minimum curvilinear embedding (MCE) analyses revealed a clear separation between the samples. Except for few documented specific differences (Fig. 2), the functional gene repertoire between the different sources appeared largely similar [2]. Our studies contribute to a better understanding of the HMA/ LMA dichotomy, provide new quantitative insights into sponge microbiology, and suggest that sponge-associated and seawater microorganisms may have most of their functional gene repertoire in common.

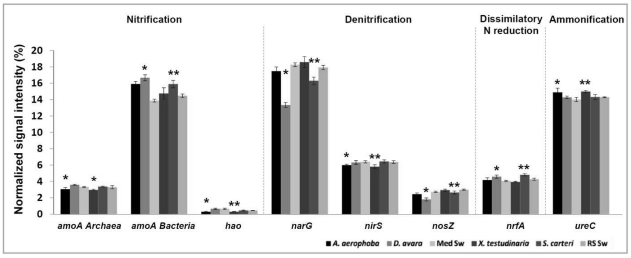

Fig. 2. Normalized average signal intensities of genes involved in nitrogen cycling. The microbial processes and corresponding genes are as follows: nitrification (archaeal and bacterial *amo*A encoding ammonia monooxygenase, *hao* for hydroxylamine oxidoreductase); denitrification (*nar*G for nitrate reductase, *nir*S for nitrite reductase, *nos*Z for nitrate reductase); dissimilatory N reduction to ammonium (*nrf*A for c-type cytochrome nitrite reductase); ammonification (*ure*C for urease). Data are presented as the mean ± SE. **: P<0.01, *: P<0.05. "Med Sw" and "RS Sw" stand for Mediterranean and Red Sea seawater.

References
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