Bacteria in the marine sponge *Pachymatisma johnstonia* – stable association or temporarily changing biocoenosis?

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Introduction

Sponges (Porifera) are sessile filter feeders known to harbour large amounts of bacteria in their tissue. The exact nature of the sponge bacteria association (symbiosis, commensalism, or parasitism/infection) is ambiguous in most cases although there is evidence for specific microbial groups within some sponges species (Fieseler et al 2004). To date several marine sponges were investigated with regard to associated bacteria, mainly focussing on mediterranean or tropical sponges. *Pachymatisma johnstonia* (Demospongia, Fig. 1) is a massive sponge which grows in the temperate waters of the North Sea (Northern Atlantic). *P. johnstonia* produces a potent bioactive glycoprotein (Pachymatismin) with antibiotic effects against Leishmaniasis. The source of this substance, either sponge-born or produced by associated bacteria, still needs to be resolved. Since most of the marine bacteria are not culturable yet, we started to identify bacteria associated with *Pachymatisma* using culture independent methods. Specimen, collected at different locations around the Orkney Isles were both analysed regarding their associated bacteria directly after sampling and after 1 year of maintenance in aquaculture on Helgoland. Molecular techniques (RISA-PCR and DGGE-PCR, 16S rDNA cloning) were used to estimate the diversity and variability of the bacterial communities associated with *Pachymatisma* specimen of different origin and to identify specific groups of bacteria associated with the sponge.

Fig. 1 ***Pachymatisma johnstonia*** **(Demospongiae, Geodiidae)**
(Picton, B.E. & Morrow, C.C., 2004. http:// www.habitas.org.uk/marinelife/porifera)

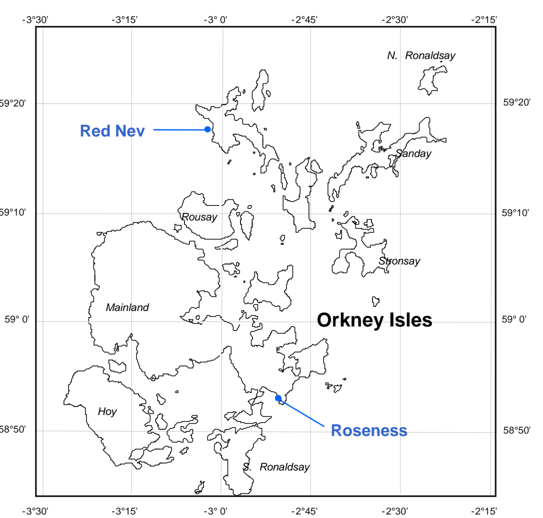

Fig. 2 The Orkney Isles (North Scotland), sampling stations are indicated by arrows

Material and Methods

The sponge specimens examined in this study were collected by SCUBA diving in 1999 and 2001 northwest (station Red Nev) and southeast (station Roseness) off the Orkney Isles (Fig. 2). They were processed immediately after collection as shown in Fig. 3. One specimen from station Red Nev (2001) was maintained for 1 year in a circulating seawater aquarium on Helgoland and processed thereafter.

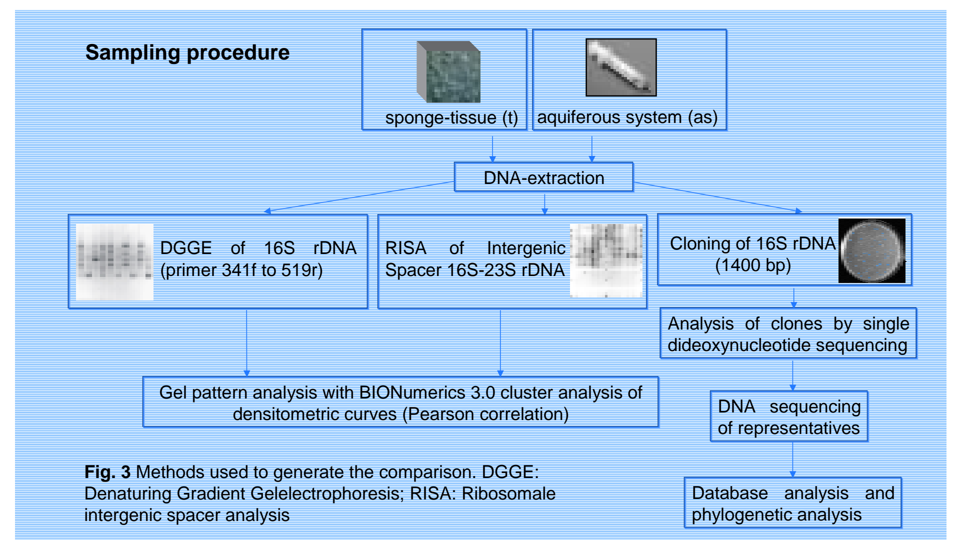

Fig. 3 Methods used to generate the comparison. DGGE: Denaturing Gradient Gelelectrophoresis; RISA: Ribosomale intergenic spacer analysis

Results

Fig. 4 Comparison of *P.johnstonia*-associated microbial communities by A: RISA-PCR analysis of intergenic spacer (16S rDNA - 23S rDNA) and B: by DGGE-PCR analysis of 193bp from the V3- region of 16S rDNA (as- aquiferous system; t- tissue). Cluster analysis based on Pearson correlation from densitometric curves. Dendrogram was constructed by applying Ward´s algorithm. (grey = cluster of the cultivation).

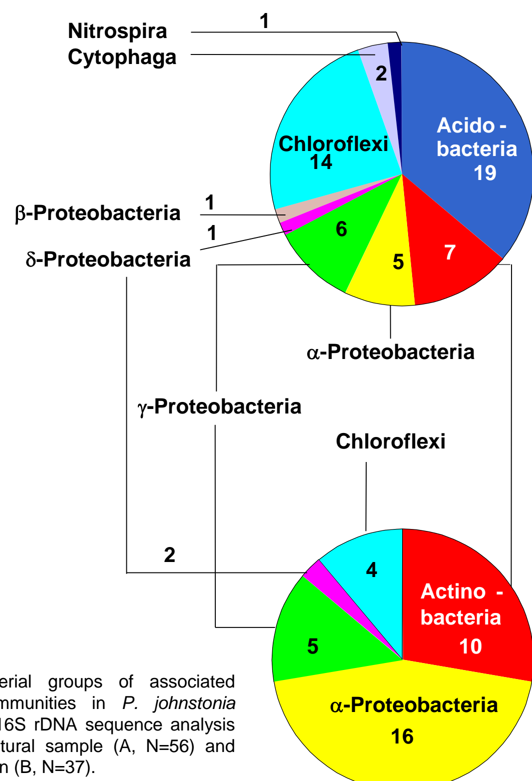

Fig. 5 Bacterial groups of associated microbial communities in *P. johnstonia* identified by 16S rDNA sequence analysis (1400 bp) natural sample (A, N=56) and after cultivation (B, N=37).

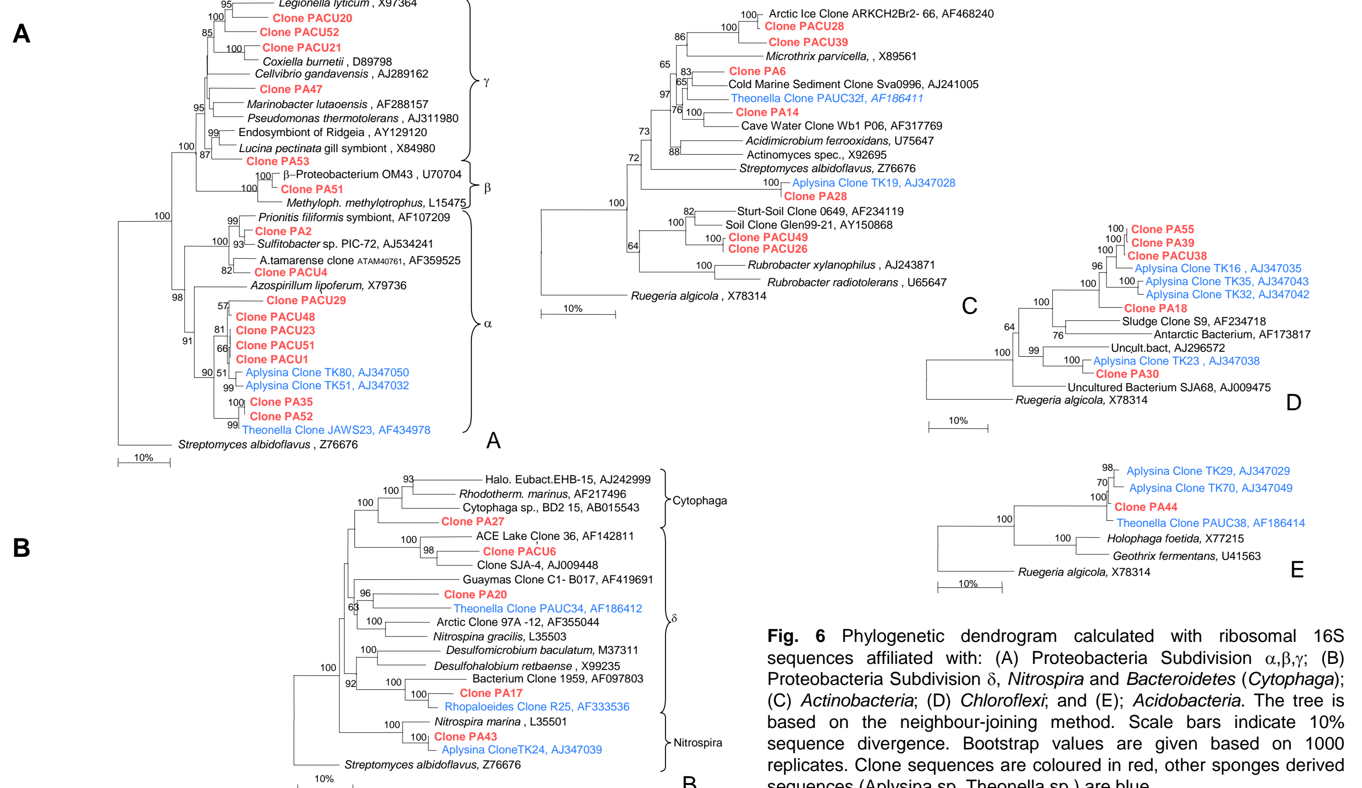

Fig. 6 Phylogenetic dendrogram calculated with ribosomal 16S sequences affiliated with: (A) Proteobacteria Subdivision α,β,γ; (B) Proteobacteria Subdivision δ, *Nitrospira* and *Bacteroidetes* (*Cytophaga*); (C) *Actinobacteria*; (D) *Chloroflexi*; and (E); *Acidobacteria*. The tree is based on the neighbour-joining method. Scale bars indicate 10% sequence divergence. Bootstrap values are given based on 1000 replicates. Clone sequences are coloured in red, other sponges derived sequences (Aplysina sp. Theonella sp.) are blue.

Conclusion

With both fingerprinting techniques we were able to show stable bacterial communities in *Pachymatisma johnstonia* by similar band patterns (RISA, DGGE, Fig. 4) in specimens of different locations and different years. In contrast after maintenance of sponge specimens in a seawater aquarium on Helgoland (1 year), the associated microbial community showed significant changes. The diversity of bacterial OTUs decreased, but still a number of shared OTUs were present. Presumably a fraction of the sponge-associated microbial community partly resides permanently in *P. johnstonia* tissue, whereas another fraction disappeared or changed. Also the 16SrRNA gene libraries of these samples evince conspicuous changes in the composition of the bacterial communities in *P. johnstonia* after cultivation. Bacteria found in this study belong to already described groups of sponge specific bacteria (Hentschel et al. 2002). In their natural habitat, main associated bacterial groups were *Acidobacteria* and *Chloroflexi*. After cultivation the number of bacterial groups decreased (from 9 to 5 groups, Fig. 5). The dominating *Acidobacteria* and several other groups disappeared; two groups increased (*Actinobacteria*, α-Proteobacteria). Only within two bacterial clusters (*Chloroflexi* and α-Proteobacteria) DNA-clones retrieved before and after cultivation were similar (Fig. 6). Presumably bacterial species composition of *Pachymatisma* is governed by the specific habitat and might be explained to some extend rather by selective enrichment of specific bacteria than by symbiosis.

References

Fieseler L., Horn M., Wagner M., and U. Hentschel (2004). Discovery of the Novel Candidate Phylum "*Poribacteria*" in Marine Sponges. Appl. Envir. Microbiol. 70: 3724

Hentschel U, Hopke J, Horn J, Friedrich A., Wagner M, Hacker J, and BS. Moore (2002). Molecular Evidence for a Uniform Microbial Community in Sponges from Different Oceans. Appl. Envir. Microbiol. 68: 4431

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