Abstract

Sponges (class porifera) are considered to be the earliest metazoans. Various sponge species are known to host dense populations of specific microorganisms which may contribute significantly to the total biomass (Vacelet, 1975). Sponge-microbe associations constructed reef buildings during various episodes of the earth history (Brunton & Dixon, 1994; Riding & Zhuravlev, 1995). Thus, the characterisation of the source organisms and the diagenetic fate of molecular cell constituents of sponges are of great interest. Recent studies have shown, that sponges and/or associated micro-organisms are prominent producers of so-called secondary metabolites (Pawlik, 1993; Haygood et al., 1999). However, since the majority of microbes eluded isolation so far, the discrete source organisms of biotechnological interesting compounds often remain unknown. Although recent gene sequence surveys contributed much to the understanding of microbial compositions in sponges (Hentschel, 2002), specific prokaryotes occuring in low densities might not have been aquired.

The present thesis deals with the qualitative and semiquantitative characterisation of microorganisms associated with boreal sponges. Applying lipid biomarker analysis, the main objective of this work was to establish a data basis for the investigation of specific sponge-associated microorganisms and their constituents. About 50 species of the sponge classes Hexactinellida and Demospongiae of worldwide distributed cold and deep-water habitats were analysed for their inventory of lipids attributed to Archaea and Bacteria. The results of hydrochemical studies, which were performed simultaneously during the sampling of most demosponges, hint for annual stable water conditions at the mid Norwegian shelf.

For comparison, about 50 cultivates of sponge-associated bacteria affiliated to the proteobacteria or the firmicutes group were analysed with regard to fatty acids (FA). α- and γ-proteobacteria predominantly synthesized saturated and monounsaturated linear C_{12}- to C_{19}-FA in genus specific distribution patterns, whereas terminal methylbranched FA prevail in the bacillus species. Various compounds, like hexadecenoic and octadecenoic acids were found in numerous bacterial taxa. In this study it was found, that the $C_{16:1}/C_{18:1}$ ratio allows for a taxonomical assignment of α- and γ-proteobacteria.

Based on numerical analysis of bacterial FA in sponges, the traditional classification of bacteriosponges and non-bacteriosponges (*sensu strictu* Reiswig) was differentiated. In particular for demosponges it was observed, that the density of bacterial cells is linked to the habitus of the host organisms. With few exceptions vast amounts of bacterial FA were observed in species of various Demospongiae-families comprising high biomass and dense tissues respectively. For the sponges examined, it was found, that the $n\text{-}C_{16:0}/n\text{-}C_{18:0}$ ratio might be used for an estimation of the bacterial density in sponge tissues.

All bacteriosponges comprise diverse FA and hopanoids often found in Gram-negative and -positive bacteria. Moreover, components presumably of bacterial origin or solely known from bacteria of other biotopes, were observed in partially high concentrations. In bacteriosponges complex isomeric mixtures of mid chain branched (mcb-)FA are prevailing. Regarding their structural peculiarities, relatives of sulfate-reducing δ-proteobacteria, β- or γ-proteobacteria, or actinomycetes are assumed as source organisms of these components. The spatial distribution of the mcb-FA within the tissue of *Geodia barretti* signifies, that they are synthesized by (facultative) anaerobic bacteria. Since mcb-FA are regarded as features of evolutionary primitive bacteria, the pronounced presence of these compounds may mirror the coevolution of sponges and associated bacteria. Moreover, compound

specific analysis of stable carbon isotopes for *G. barretti* indicated the utilisation of mcb-FA for the synthesis of "demospongic acids".

In bacteriosponges the appearance of non-isoprenoidal glycerol mono ether lipids suggests the presence of sulfate-reducing δ-proteobacteria or clostridia while long chain dicarboxylic acids most probably originate from anaerobic clostridia. Thus several lipid classes indicate the presence of oxygen-depleted compartments within the tissues of these sponges.

For the first time the association between Archaea and numerous hexactinellids and several demospongiae was established by lipid biomarker analysis. The distribution of etherbound acyclic biphytanes and homologues with up to three cycloalkylrings hints for the dominance of crenarchaeota. However, compared to the crenarchaeotal sponge symbiont *Cenarchaeum symbiosum* previously described (Preston et al., 1996, DeLong et al. 1998), a higher relative abundance of the acyclic biphytanes was found in the sponges studied, suggesting an additional contribution by euryarchaeotes. Archaea were predominantly observed in sponges poor in bacteria. For the axinellid non-bacterio-sponge *Phakellia ventilabrum* the biomass of sponge-associated Archaea was estimated based on nominal cell weight. Compared with the bacterial cell density in *P. ventilabrum* calculated by others using DAPI staining and microscopic counting, it may be assumed that Archaea – though most probably existing in various sponge taxa – in *P. ventilabrum* constitute a relatively small fraction of sponge-associated microbial community.

The results of this thesis may serve as a basis for microbiological studies regarding the search for novel biotechnological interesting compounds. Lipid biomarker analysis proved to be an effective tool for a qualitative and quantitative evaluation of sponge-associated microorganisms. Using this concept specific endobiotic groups like Archaea, clostridia, sulfate-reducing proteobacteria or actinomycetes, which lack profound documentation so far, were detected in the sponges investigated. Thus, the relevance of the biomarker analysis of sponges for the specific search for secondary metabolites could be evidenced.