MINIREVIEW

Marine sponges as microbial fermenters

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Abstract

The discovery of phylogenetically complex, yet highly sponge-specific microbial communities in marine sponges, including novel lineages and even candidate phyla, came as a surprise. At the same time, unique research opportunities opened up, because the microorganisms of sponges are in many ways more accessible than those of seawater. Accordingly, we consider sponges as microbial fermenters that provide exciting new avenues in marine microbiology and biotechnology. This review covers recent findings regarding diversity, biogeography and population dynamics of sponge-associated microbiota, and the data are discussed within the larger context of the microbiology of the ocean.

Sponges as animal hosts

Sponges (phylum *Porifera*) are among the oldest metazoan animals, with a fossil record dating back more than 580 million years to the Precambrian. Sponges contribute significantly to, and sometimes even dominate, the tropical reef fauna in terms of biomass, but are also found in polar and deep oceans and in freshwater lakes and streams. Eighty-five percent of the 6000 formally described living species belong to the class *Demospongiae* (demosponges), with the other species being represented by the classes *Hexactinellida* (glass sponges) and *Calcarea* (calcareous sponges) (Hooper & van Soest, 2002). The growth habits of sponges encompass various shapes (e.g. encrusting, rope, ball, tube, barrel, vase), colours (e.g. white, yellow, green, blue, purple, brown, black) and sizes (a few millimetres to nearly two metres), and can be quite variable in response to environmental conditions (Fig. 1) (Brusca & Brusca, 1990). Owing to the paucity of consistent morphological parameters, a taxonomic classification of sponges has been difficult. Moreover, because the molecular markers are frequently limited in resolution and the data do not always agree with classical taxonomy, this important field is still in its infancy (Borchiellini *et al.*, 2004). As filter feeders, sponges are capable of turning over many thousands of litres of water per day. Prokaryotic microorganisms, as well as nano- and picoeukaryotes, are the most important components of the sponge diet (Pile, 1997; Ribes *et al.*, 1999). Following capture, food particles are moved into the mesohyl interior and are phagocytosed by amoeboid sponge cells, termed archaeocytes (Fig. 2). Particle uptake by sponges appears to be highly efficient but probably largely unselective for a given particle size range (Pile, 1997; Ribes *et al.*, 1999 and references cited herein).

Microbial communities of sponges

Despite the fact that sponges feed on microorganisms, large numbers of extracellular bacteria populate the mesohyl matrix of many demosponges. These types of sponge have been termed 'bacteriosponges' or 'high-microbial-abundance sponges' (Vacelet & Donadey, 1977; Hentschel *et al.*, 2003). However, the mesohyl of other sponges that coexist in the same habitat are essentially devoid of microorganisms ('low-microbial-abundance sponges') (Fig. 3). In the bacteriosponges, bacterial population densities may reach 10^8–10^{10} bacteria per gram of sponge wet weight, exceeding seawater concentrations by 2–4 orders of magnitude, whereas in the low-microbial-abundance sponges, they are within the range of natural seawater (10^5–10^6 bacteria per

Fig 1. The two high-bacterial-abundance demosponges *Xestospongia muta* (back) and *Agelas* sp. (front) on Caribbean reefs (photograph by Joseph Pawlik, Wilmington, USA).

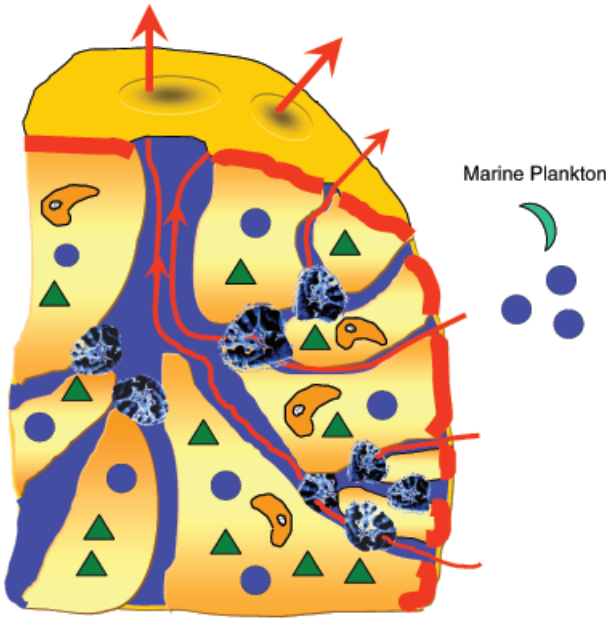

Fig. 2. Schematic representation of a typical bacteriosponge. The aquiferous canal system is shown in blue. The movement of seawater as it enters the ostia, passes through the choanocyte chambers and leaves the sponge through the exhalant canals is demonstrated by red arrows. Single amoeboid sponge cells responsible for phagocytosis and digestion are shown in orange. The red shading on the sponge surface shows the cyanobacteria on the light-exposed surfaces. Current understanding of microbe–sponge interactions: green triangles represent the fraction of microorganisms that are presumably vertically transmitted through the reproductive stages. Blue circles symbolize the fraction of microorganisms that are taken up from seawater. Once inside the mesohyl, they are either digested quickly, serving as a food source, or they may become part of the sponge-specific microbiota. Green crescent-shaped symbols represent seawater bacteria that probably pass through the sponge unprocessed.

gram of sponge wet weight) (U. Hentschel, unpublished data). Because the high-microbial-abundance sponges are typically larger than their low-microbial abundance counterparts whose mesohyl is essentially devoid of bacteria, it is tempting to speculate that the presence of internal microbial biomass contributes to their larger size. The Caribbean great barrel sponge *Xestospongia muta* serves as a suitable example to illustrate this point (Fig. 1). As the largest known sponge species, *X. muta* may reach about 6–8 ft (2 m) in height. With a bacterial population density of 8×10^9 microorganisms per gram of wet weight a single individual, conservatively estimated at 10 kg wet weight, will hold a bacterial population size of nearly 10^{14} microorganisms (U. Hentschel, unpublished data). These numbers underline the ecological relevance of sponge-associated microbiota for tropical reef ecosystems.

The microbial distribution within a typical sponge residing in the photic zone follows a general pattern. The outer, light-exposed layers are populated by photosynthetic microorganisms, while the internal mesohyl contains a complex mixture of heterotrophic and probably also autotrophic bacteria. The vast majority of microorganisms are located extracellularly in the mesohyl matrix, where they appear to be homogenously mixed. In some sponges, bacteria are found within host bacteriocytes (Vacelet & Donadey, 1977) and even within host nuclei (Friedrich *et al.*, 1999). From a nutritional perspective, sponges should be suitable niches for microorganisms compared with the nutrient-poor seawater, particularly in tropical regions. As ammonia is an end product of the host metabolism, it is likely to be available as a nitrogen source, while carbohydrates and amino acids should be available as a result of the extensive phagocytosis of the host. Indeed, bacteria within sponge tissues are clearly metabolically active, as evidenced by bright fluorescence hybridization (FISH) signals, which serve as indirect indicators of cellular rRNA content. Provided that the bacteria can avoid being digested, the mesohyl should be a stable and nutritionally rich habitat.

Until several years ago, the microbial communities of sponges were only identified by electron microscopy (Vacelet & Donadey, 1977; Wilkinson, 1978; Rützler, 1990). While early works indicated high microbial diversity and the

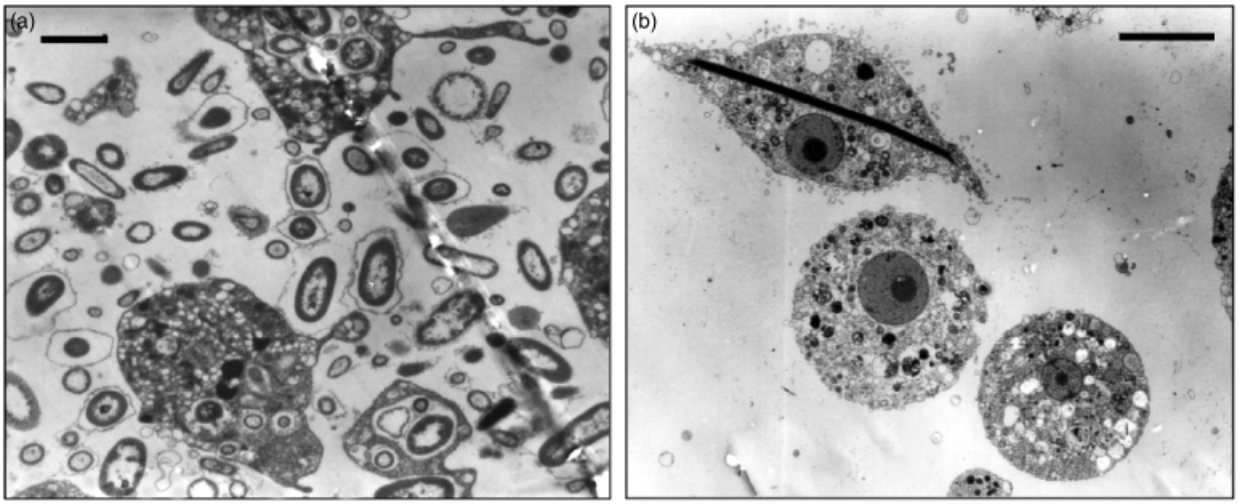

Fig. 3. Transmission electron micrographs of the high-bacterial-abundance sponge *Xestospongia muta* (a) and the low-bacterial-abundance sponge *Callyspongia vaginalis* (b) from Key Largo, USA. The size bars are 1.5 and 5 μm, respectively (micrograph by Markus Wehrl, Würzburg, Germany).

presence of unusual microorganisms, it was not until the application of molecular tools, specifically 16S rRNA gene library construction, FISH, and denaturing gradient gel electrophoresis (DGGE), that more precise insights into the microbial community composition could be gained. Nearly a dozen studies have now been undertaken, with sometimes strikingly congruent results (for recent reviews, see Hentschel *et al*., 2003; Imhoff & Stöhr, 2003; Hill, 2004). 16S rRNA gene library construction using universal bacterial PCR primers revealed a common microbial signature in many sponges that is phylogenetically complex yet highly sponge-specific and distinctly different from that of marine plankton (Fig. 4). 16S rRNA gene phylotypes affiliated with the phyla *Acidobacteria*, *Chloroflexi*, *Actinobacteria*, *Proteobacteria* (*Alpha-*, *Gamma-*, *Delta-*), *Nitrospira*, *Cyanobacteria*, and *Bacteriodetes,* as well as a cluster of uncertain affiliation now recognized as the *Gemmatimonadetes* (U. Henschel, unpublished data) were recovered by this approach. In addition, a crenarchaeal lineage was documented in *Axinella mexicana* and other axinellid sponges (Preston *et al*., 1996; Margot *et al*., 2002), and a new eubacterial candidate phylum 'Poribacteria' was recently discovered in verongid sponges (Fieseler *et al*., 2004). The fact that the apparently sponge-specific phylotypes are present below detection levels or may even be absent from seawater, sediment and other environments indicates highly selective conditions for microbial existence within the sponge mesohyl.

As common patterns are beginning to emerge, so are the exceptions. Among three Australian temperate reef sponges investigated by (Taylor *et al*., 2004), only one (*Cymbastela concentrica*) showed convincing evidence for sponge-specific phylotypes. In contrast, the microbial profiles of the co-

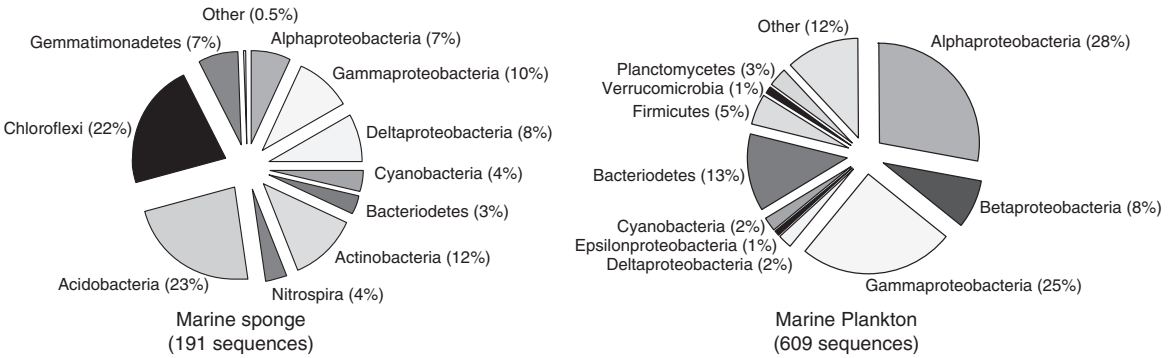

Fig. 4. 16S rRNA gene sequences derived from marine sponges compared with those from marine bacterioplankton based on PCR amplification with universal bacterial primers (summarized from Hagstrom *et al*., 2002; Hentschel *et al*., 2002). Note that the abundances of phylotypes in gene libraries cannot be extrapolated to *in situ* abundances. Lineages such as the *Candidatus* phylum 'Poribacteria' and the *Archaea* are not represented because of mismatches in the primer regions.

occurring *Callyspongia* spp. and *Stylinos* spp. were highly similar to that of seawater. Because the Caribbean *Callyspongia vaginalis* and *Callyspongia plicifera* belong to the low-bacterial-abundance group (Fig. 3b), it is possible that the Australian *Callyspongia* spp. and possibly *Stylinos* spp. might also belong to the low-bacterial-abundance group. Webster *et al.* (2004) performed a molecular diversity analysis of five Antarctic sponges. The microbial diversity within these sponges was substantially lower than that of seawater, the phylotypes were most closely related to previously retrieved sequences from polar (nonsponge) environments, and monophyletic clusters, as described by Hentschel *et al.* (2002), were not evident. Whether these patterns are a result of the extreme Antarctic conditions or whether all five species belong to the low-bacterial-abundance group remains to be seen. Another study addressed the microbial community composition of a freshwater sponge (Gernert *et al.*, 2005). The microbial diversity of *Spongilla lacustris* was comparably small and largely resembled that of surrounding lake water. Two novel, deeply rooting alphaproteobacterial lineages were identified that may be specific to their sponge host. Because the mesohyl of *S. lacustris* was virtually bacteria-free, as evidenced by electron microscopy, the authors propose that this is the reason for the lack of sponge-specific microbiota.

The discovery of large microbial communities in sponges expands the known panel of marine symbiotic model systems that are based on the highly specific associations between one symbiont and one invertebrate host. Well-known examples are the two-partner mutualisms of photosynthetic algae (*zooxanthellae*) with corals, anemones and giant clams, of chemolithoautotrophic bacteria with hydrothermal vent clams and tubeworms, and of heterotrophic bacteria with bioluminescent squids and ship-boring bivalves (Hentschel *et al.*, 2000; Steinert *et al.*, 2000). Collectively, the microbial symbionts confer upon their hosts the potential to exploit an impressive metabolic repertoire including photosynthetic carbon fixation, nitrification, anaerobic metabolism, bioluminescence, and secondary metabolite production. Consequently, primary production by symbiotic microbes can have major ecological ramifications for the host, enabling the habitation of nutrient-poor tropical waters as well as toxic deep-sea vent environments. With respect to sheer microbial biomass and phylogenetic complexity, the commensal microbiota of sponges is unmatched in the realm of marine symbioses, rivalling those of cattle rumen and other intestinal microbial ecosystems. While these large numbers can most certainly be attributed to favourable nutritional conditions within the sponge, their putative functions are not easily revealed. Possible symbiotic functions include the exchange of primary metabolites contributing to nutrition, and the production of secondary metabolites for chemical defence purposes. The following section discusses specific lineages that have been identified as members of the sponge-associated microbiota.

Specific lineages

Cyanobacteria of the outer surfaces

Cyanobacteria are probably the most abundant photosyn thetic organisms on Earth, with the *Synechococcus/Prochlorococcus* clade representing the single most dominant clade in the world's oceans. Their presence has been confirmed in at least 26 *Demospongiae* families and in at least 17 *Calcarea* families. Some of these sponge–cyanobacteria associations represent the most prominent benthic organisms in given areas. Cyanobacteria are usually responsible for the colouration of their sponge host, and changes in phycobiliprotein ratios can mean that a given species of sponge host is yellow/green in bright light and red/brown in low light (Usher *et al.*, 2004a). Even though they are typically found on the outer light-exposed surfaces, cyanobacteria can also be distributed throughout the inner core of the sponge. This mutually beneficial association is thought to be one of the oldest microbe–metazoan interactions, and is speculated to have dominated over hard corals during periods of the Paleozoic and Mesozoic.

Molecular studies are now beginning to clarify the taxonomy and phylogeny of cyanobacterial symbionts of sponges. Several 16S rRNA studies have shown that the colloquially known '*Aphanocapsa feldmannii*-like' symbionts are in fact *Synechococcus* species (Hentschel *et al.*, 2002; Gómez *et al.*, 2004; Usher *et al.*, 2004b). The '*Aphanocapsa feldmannii*'/'*Synechococcus feldmannii*'-type symbionts consist of at least four closely related lineages (Usher *et al.*, 2004a). A phylogenetically distinct lineage was identified in *Chondrilla nucula* and was given the name of *Candidatus* 'Synechococcus spongiarum' (Usher *et al.* 2004b) (Fig. 5a, b). Steindler *et al.* (2005) significantly expanded the existing dataset and showed the presence of a monophyletic '*Synechococcus spongiarum*' clade in a total of 18 sponge species from Australia, the Caribbean, Mediterranean, Red Sea, and Zanzibar that is phylogenetically clearly distinct from the nearest free-living *Synechococcus* relative (Fig. 5b, c).

Such detailed information on the *Synechococcus* symbiont clade allows speculations as to how this lineage was formed. Vertical transfer through the larval stages has been demonstrated using electron microscopy and molecular tools (Usher *et al.*, 2001, 2005; Oren *et al.*). Surprisingly, the members of *Synechococcus* clade lack cospeciation with their sponge hosts, a feature that one might have expected from many other evolutionarily ancient symbiotic model systems. It is therefore conceivable that a free-living form of the *Synechococcus* sponge symbionts exists in the ocean, albeit at very low concentrations, and is selectively enriched upon

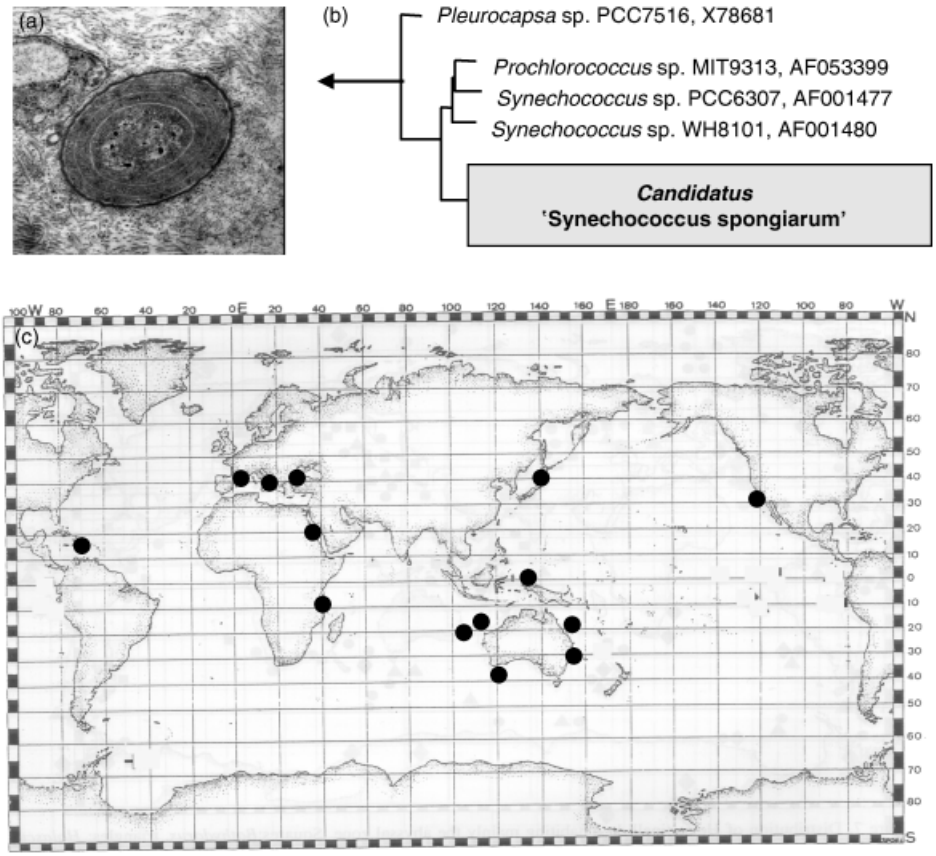

Fig. 5. Electron microscopy (a), phylogenetic position (b) and geographical distribution (c) of the *Candidatus* 'Synechococcus spongiarum' clade of sponge symbionts (summarized from Usher *et al.*, 2004a and Steindler *et al.*, 2005).

encounter with a suitable sponge. The *Synechococcus* sponge lineage might then be considered as a 'sponge ecotype' by analogy to the 'shallow' and 'deep' *Synechococcus* ecotypes that have been described for planktonic cyanobacteria (Ferris & Palenik, 1998). However, as long as environmental samples fail to turn up a free-living form of the *Synechococcus* sponge symbionts, the evolutionary history of this clade remains to be elucidated.

A filamentous cyanobacterium, *Oscillatoria spongeliae*, is housed by many *Dysidea* sponges. In contrast to the *Synechococcus* clade of sponge symbionts, the *O. spongeliae* symbionts exhibit a high degree of host specificity, with each sponge species harbouring its own symbiont strain (Thacker & Starnes, 2003; Ridley *et al.*, 2005). As a result, host and symbiont phylogenies largely mirror each other, with the exception of a single symbiont-switching event (Ridley *et al.*, 2005). The fact that filamentous oscillatoriae are absent from the seawater microbiota probably reduces the extent of mixing of phylogenetic lineages and could therefore be the main reason why cospeciation could be documented. In light of the evolutionary age of sponges and cyanobacteria this close association is a particularly exciting find.

Other cyanobacterial symbionts of sponges include *Aphanocapsa raspaigellae, Synechocystis trididemni* and *Prochloron*. As with many cyanobacterial symbionts, their phylogeny is uncertain. Additional, so far unnamed, cyanobacterial symbionts include a 4 μm symbiont of *Mycale hentscheli*, related to *Cyanobacterium stanieri*, and a 1 μm symbiont of *Cymbastela marshae,* related to *Oscillatoria rosea*.

Microorganisms of the inner core

Archaea

The *Archaea* are the second best-understood sponge-associated microbial lineage next to the cyanobacteria. Marine sponge-associated archaea were originally discovered by Preston *et al.* (1996), who reported on the consistent association of archaeal 16S rRNA gene sequences with *Axinella mexicana*. Metagenomic library construction from enriched sponge microbial consortia of *A. mexicana* revealed the coexistence of at least two closely related lineages in a single host sponge (Schleper *et al.*, 1998). Originally described as *Cenarchaeum symbiosum*, closely related

phylotypes have since been found in the Australian sponge *Rhopaloeides odorabile*, in Mediterranean sponges of the genus *Axinella*, and in eight different sponge species from the Korean coast (Webster *et al.*, 2001a; Margot *et al.*, 2002; Lee *et al.*, 2003). Terminal restriction fragment length polymorphism
(T-RFLP) analysis in the latter study revealed that the phylogenetic diversity within each sponge is low and, following sequencing and phylogenetic tree construction, it was possible to appoint the sequences to five sponge-specific sequence clusters (Lee *et al.*, 2003). The worldwide distribution of the sponge-specific *C. symbiosum* clade resembles the similarly cosmopolitan distribution of the free-living marine crenarchaeote group I, to which they are most closely related. The wide distribution suggests that a free-living form of this archaeal sponge symbiont clade exists which is able to persist in sponges following uptake by filtration.

Proteobacteria

Molecular phylogenetic studies have provided a complex picture of proteobacterial diversity within sponges and sometimes allow a first glimpse into their metabolism within the sponge habitat. Two sponge-specific alphaproteobacterial sequence clusters were recovered from several sponges that are most closely related to an uncultured marine coastal picoplankton sequence (Hentschel *et al.*, 2002). A sponge-specific gammaproteobacterial cluster showed closest homology to the ammonia-oxidizing *Nitrosococcus* clade, while phylotypes belonging to the *Nitrosomonas eutropha/europaea* lineage have been recovered from various mangrove sponges (Diaz *et al.*, 2004). The coordinated metabolism of *Nitrosococcus* and/or *Nitrosomonas* as ammonia-oxidizing bacteria (AOB), together with *Nitrospina* and/or members of the phylum *Nitrospira* as nitrite-oxidizing bacteria (NOB) might be responsible for the process of nitrification in sponges. Nitrification describes the catalyzation of ammonia (NH_3) to nitrite (NO_2^-) by AOB and subsequently to nitrate (NO_3^-) by NOB for energy purposes. Indeed, nitrate excretion has already been documented on several occasions, making nitrification a likely scenario (Davy *et al.*, 2002). The capacity of microbial dehalogenation was explored in aplysinid sponges, which are known as a rich source for halogenated secondary metabolites (Ahn *et al.*, 2003). Indeed, the presence of anaerobic dehalogenating microorganisms, specifically representatives of the *Deltaproteobacteria* and *Chloroflexi*, could be demonstrated in *A. aerophoba* along with the presence of several dehalogenase gene fragments. Moreover, enrichment cultures from sponge tissues revealed that the sponge microbial community is capable of dehalogenating a wide range of organohalide compounds. Aplysinid sponges, as well as the many other sponge genera with similar chemistry, may thus be considered as an underexplored reservoir for dehalogenating microorganisms.

Several cultivation studies have produced a multitude of sponge-derived *Proteobacteria*, many of which are conveniently presented on the Harbor Branch Marine Microbial Database (HBMMD) (Gunasekera *et al.*, 2005). More than two-thirds of the thousands of cultivated isolates from deep-water and boreal sponges and other invertebrates belonged to the *Alphaproteobacteria* and *Gammaproteobacteria* (Graeber *et al.*, 2004; Sandell *et al.*, 2004), while *Gammaproteobacteria* dominated among the nearly 2000 isolates from *Halichondria panicea* (Imhoff & Stöhr, 2003). The dominance of the alphaproteobacterial strain MBIC3368 in culture collections from marine sponges is particularly noteworthy. This strain has been recovered from at least eight different sponge sources and can sometimes make up almost the entire culturable fraction (Webster & Hill, 2001). MBIC3368 strains appear to be exquisitely adapted to survive on and within sponge hosts and their reported bioactivity and mucoid consistency might serve to prevent phagocytosis by sponge cells (Hentschel *et al.*, 2001; Thiel & Imhoff, 2003). Moreover, the alphaproteobacterial strain MBIC3368 is so far the only isolate that has been recovered by both cultivation and 16S rRNA-based approaches (Thoms *et al.*, 2003). However, the vast majority of sponge-specific microbial phylotypes recovered by molecular approaches are still refractory to cultivation (Lopez *et al.*, 1999; Webster *et al.*, 2001c; Olson & McCarthy, 2005). The discrepancy between cultivation-dependent and cultivation-independent approaches is a well-known phenomenon in environmental microbiology, and bridging this gap poses one of the biggest challenges in this field.

Actinobacteria

The identification of *Actinobacteria* within marine sponges is of great interest, because representatives of this phylum are prolific secondary metabolite producers, which has obvious implications for natural products and drug discovery. *Actinobacteria* have been recovered from the sponge *Rhopaloeides odorabile* using both cultivation-independent and cultivation-dependent approaches (Webster *et al.*, 2001c). Moreover, three sponge-specific actinobacterial clusters were described in *Theonella swinhoei* and *Aplysina aeropoba*, and their known distribution has recently been extended to *Scleritoderma* spp. from deeper waters and to *Xestospongia* spp. (Hentschel *et al.*, 2002; Montalvo *et al.*, 2005; Olson & McCarthy, 2005). The sponge-specific actinobacteria are members of the *Acidimicrobiae*, whose closest culturable representatives are *Microthrix parvicella* and *Acidimicrobium ferreoxidans*. These iron-oxidizing acidophiles are known from activated sludge and geothermal hot springs, respectively, and have been notoriously difficult to

cultivate. Directed cultivation efforts have yielded bioactive *Salinospora* isolates from the Australian sponge *Pseudoceratina clavata* that were previously only known from marine sediments (Kim *et al.*, 2005).

The candidate phylum 'Poribacteria'

The novel *Candidatus* phylum 'Poribacteria' appears to be specific to or at least enriched in marine demosponges, as representatives have so far not been found in the environment (Fieseler *et al.*, 2004). The 'Poribacteria' are characterized by cell compartmentation in the form of an unusual nucleoid-like structure. Nucleoid-containing bacterial morphotypes were identified in sponges by electron microscopy in early studies (Hentschel *et al.*, 2003), and more recently documented by electron microscopy by Friedrich *et al.* (1999), and by Fuerst *et al.* (1999), who also documented the presence of DNA in the nucleoid-like compartments. The morphological diversity of these unusual bacteria encompasses short and long rod-shaped cells as well as D-shaped cells. The cell walls are typical of gram-negative cell walls, but S-layer structures and bleb-like extrusions of the outer membrane have also been noted. The application of FISH with specific probes confirmed the presence of remarkably high numbers of 'Poribacteria' in sponges (Fieseler *et al.*, 2004).

Other lineages

Several other less well-known phyla abound in sponge-derived 16S rRNA gene libraries, such as the *Chloroflexi* (formerly green nonsulphur bacteria), which is closely related to the SAR202 lineage, the *Acidobacteria*, mostly known from terrestrial soils, and the newly described phylum *Gemmatimonadetes* (U. Henschel, unpublished data). Certainly much diversity is still to be discovered in these enigmatic marine microbial communities.

Stability of sponge-associated microbial communities

Temporal, intraspecies and biogeographical variability

Temporal variability in marine sponge-associated microbial communities has been examined for field-based (Sandell *et al.*, 2004; Taylor *et al.*, 2004) and aquarium-based (Friedrich *et al.*, 2001) populations of sponges. In the latter study, a stable relationship was seen over 11 days between the sponge *Aplysina aerophoba* and its resident bacteria. Neither total cell numbers nor community composition (as assessed by molecular and microscopy methods) changed appreciably during incubation in untreated seawater (Friedrich *et al.*, 2001). Similarly, in natural populations of three sponges from southeastern Australia, temporal variability was minor (Taylor *et al.*, 2004). DGGE-based community fingerprints revealed the existence of apparently highly stable bacterial communities in the demosponges *Callyspongia* sp., *Cymbastela concentrica* and *Stylinos* sp. over the course of one year. In addition, variability on a shorter (days to weeks) timescale was shown to be minimal for *C. concentrica* (Taylor *et al.*, 2004). In another investigation of temporal variability in a sponge–bacteria association, Webster and Hill (2001) demonstrated that, over four consecutive seasons, the previously mentioned alphaproteobacterial isolate MBIC 3368 dominated the culturable community of the Great Barrier Reef sponge *Rhopaloeides odorabile*. However, given that the culturable component did not exceed 0.8% of the total microbial community, and that *R. odorabile* is known to contain a highly diverse range of microorganisms, it is not yet possible to make assertions about the overall community stability in this host.

Several recent studies have sought to investigate intraspecies variability within individual hosts or host species. In the Australian sponges *Callyspongia* sp., *Cymbastela concentrica* and *Stylinos* sp., variability in community composition (as inferred from 16S rRNA-DGGE banding patterns) was minimal both within and among replicate individuals of a given host species (Taylor *et al.*, 2004). What minor variations did occur could generally be attributed more to differences among, rather than within, individual sponges. Similarly low levels of variability among conspecific hosts at a single location were reported for several Antarctic sponges, again using DGGE to describe community composition (Webster *et al.*, 2004). This study found essentially the same result when the comparison was extended to different Antarctic locations (spanning a distance of *c.* 10 km); bacterial communities differed among host species but were highly consistent within the same species, even at different collection locations. Eukaryote communities (specifically, diatoms and dinoflagellates) were also monitored and appeared to be somewhat more variable than were the bacterial communities.

The first study on biogeographical variability (hundreds to thousands of kilometres) comprised a comprehensive phylogenetic analysis of bacteria associated with the distantly related sponges *Theonella swinhoei* (from the western Pacific) and *Aplysina aerophoba* (from the Mediterranean) (Hentschel *et al.*, 2002). These data, based on more than 190 sponge-derived 16S rRNA gene sequences from *T. swinhoei*, *A. aerophoba* and *Rhopaloeides odorabile*, suggested a high degree of uniformity among sponge-associated microbial communities, regardless of host or location. The second study to explicitly examine biogeographical variability in sponge-associated bacteria employed 16S rRNA-based DGGE; here, Taylor *et al.* (2005) examined the sponge *Cymbastela concentrica* at numerous sites along the eastern

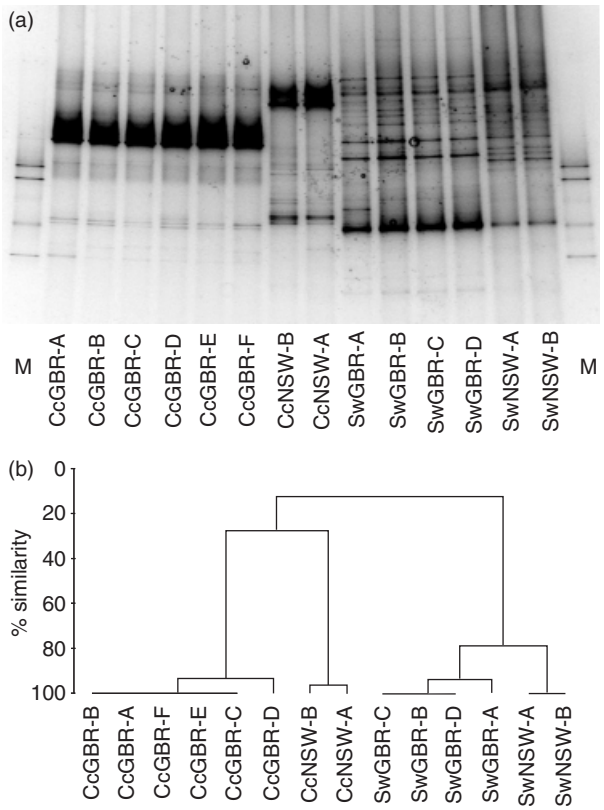

Fig. 6. Bacterial community composition in *Cymbastela concentrica* (Cc) and seawater (Sw) from a Great Barrier Reef (GBR) location and a New South Wales (NSW) location. 16S rDNA-denaturing gradient gel electrophoresis gel (a) and cluster diagram (b) representing the presence or absence of bands in (a). [Modified from (Taylor *et al.*, 2005), with kind permission of Blackwell Publishing.]

Australian coast. DGGE banding patterns (and inferred community composition) varied little over a 500 km portion of the temperate range of this sponge. In contrast, *C. concentrica* from a distant tropical (Great Barrier Reef) location appeared to contain a very different bacterial community compared with its temperate counterparts (Fig. 6). Interestingly, the bacterial community in surrounding seawater varied much less between the two areas. Possible explanations for these observations include cryptic speciation of the host sponge (i.e. the sponges from the two locations could be genetically distinct), environmental factors such as light and temperature, which change with latitude, and adaptation to geographically separated hosts (with subsequent speciation). To summarize, it appears that sponge-associated microbial communities are stable within individuals and through time (at least at the level of resolution achieved in the various studies), and it can be conservatively stated that specific subsets of the overall community occur consistently within the same sponge species from different locations.

Effects of experimental perturbation

Further insights into the stability of sponge–microbe associations have been obtained by experimentally stressing the host sponge in order to assess any resulting changes in the microbial community. In one of the first experiments of this kind, exposure of the Great Barrier Reef sponge *R. odorabile* to increasing concentrations of copper elicited a significant response in the resident microbial community (Webster *et al.*, 2001b). Bacterial numbers, as determined by culturing and FISH, both decreased following exposure, as did bacterial diversity. Likewise, archaea, which are normally abundant in *R. odorabile*, were no longer detected after 14 days of 'medium' exposure. These results indicate that many of the microorganisms present are sensitive to outside stress, or at least to free copper ions. Given the risk of copper contamination from terrestrial sources and antifouling paints to coral reef animals such as sponges, it was suggested that the microbial community may represent a sensitive bioindicator for assessment of copper pollution.

A seemingly more resistant microbial community appears to reside in the Mediterranean sponge *Aplysina aerophoba*. Neither starvation nor exposure to antibiotics for 11 days caused major changes in community composition among aquarium-maintained sponges (Friedrich *et al.*, 2001). Furthermore, when the closely related species *A. cavernicola* was experimentally transplanted from its natural depth of > 40 m to depths of 7–15 m, the associated bacterial community remained largely unchanged, despite often substantial degradation of host sponge tissues (Thoms *et al.*, 2003). Interestingly, of the permanent subset of the bacterial community – as assessed by 16S rRNA-DGGE analysis – many sequences grouped with the aforementioned sponge-specific clusters, whereas the variable bacterial fraction was typically related to common seawater bacteria. Another transplantation experiment, again involving an aplysinid sponge, involved moving *A. fistularis* and *Ircinia felix* from their natural depth of 4 m to depths exceeding 100 m (Maldonado & Young, 1998). Of those sponges that survived at 100 m, individuals of *I. felix* lost their cyanobacterial associates but underwent a concomitant change in morphology, presumably to alter feeding habits and compensate for the loss of symbiont-derived nutrition. In contrast, cyanobacteria remained abundant in *A. fistularis* at this depth. The effects of light and depth on symbiotic cyanobacteria were also investigated in the tropical sponge *Dysidea granulosa* (Becerro & Paul, 2004). Cyanobacterial abundance (as estimated by the tissue concentration of chlorophyll *a*) decreased significantly following transplantation of sponges to a deeper site, and total light reductions resulting from experimental shading had a similar effect.

Conclusions and outlook

(1) The discovery of huge microbial diversity in marine sponges provides unprecedented research opportunities. In particular, the development of new and innovative cultivation strategies holds great potential to access the microbial lineages that are so far underrepresented in culture. Metagenomic library construction will continue to open new avenues to describe diversity, and, more importantly, to recover functional genes and operons from the so far uncultivated fraction of sponge-associated microbiota. This will also improve our understanding of the physiological roles of sponge-associated microorganisms and their role in a possibly symbiotic association with their sponge host, which is clearly an important area for future research.
(2) Understanding variability and specificity is a fundamental goal when examining any microbe–host association. Based on the discussed data, it is likely that the stability of microbial communities may vary among host sponges and also among the different microorganisms under investigation. Moreover, different stressors will almost certainly affect microbial community composition in different ways. Given that marine sponges are exposed to immense volumes of seawater, documentation of such variability is crucial in order to understand microbial population dynamics in sponges.
(3) Microbial biogeography is a topic of much current interest, yet there is no clear consensus on whether the paradigm 'everything is everywhere' holds true for the microbial communities of sponges, or whether bacterial speciation does indeed occur as might be suggested by some of the results discussed above. The study of community stability among sponge-associated microorganisms is in its infancy, and, clearly, much remains to be learned about the host-related, environmental and geographical factors that will be important for the stability and specificity of the sponge–microbe association.

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