J Ind Microbiol Biotechnol (2006) 33: 545–551
DOI 10.1007/s10295-006-0123-2

REVIEW

Guangyi Wang

Diversity and biotechnological potential of the sponge-associated microbial consortia

Received: 18 November 2005 / Accepted: 22 February 2006 / Published online: 22 April 2006

Abstract Sponges are well known to harbor diverse microbes and represent a significant source of bioactive natural compounds derived from the marine environment. Recent studies of the microbial communities of marine sponges have uncovered previously undescribed species and an array of new chemical compounds. In contrast to natural compounds, studies on enzymes with biotechnological potential from microbes associated with sponges are rare although enzymes with novel activities that have potential medical and biotechnological applications have been identified from sponges and microbes associated with sponges. Both bacteria and fungi have been isolated from a wide range of marine sponge, but the diversity and symbiotic relationship of bacteria has been studied to a greater extent than that of fungi isolated from sponges. Molecular methods (e.g., rDNA, DGGE, and FISH) have revealed a great diversity of the unculturable bacteria and archaea. Metagenomic approaches have identified interesting metabolic pathways responsible for the production of natural compounds and may provide a new avenue to explore the microbial diversity and biotechnological potential of marine sponges. In addition, other eukaryotic organisms such as diatoms and unicellular algae from marine sponges are also being described using these molecular techniques. Many natural compounds derived from sponges are suspected to be of bacterial origin, but only a few studies have provided convincing evidence for symbiotic producers in sponges. Microbes in sponges exist in different associations with sponges including the true symbiosis. Fungi derived from marine sponges represent the single most prolific source of diverse bioactive marine fungal compounds found to date. There is a developing interest in determining the true diversity of fungi present in marine sponges and the nature of the association. Molecular methods will allow scientists to more accurately identify fungal species and determine actual diversity of sponge-associated fungi. This is especially important as greater cooperation between bacteriologists, mycologists, natural product chemists, and bioengineers is needed to provide a well-coordinated effort in studying the diversity, ecology, physiology, and association between bacteria, fungi, and other organisms present in marine sponges.

Keywords Marine sponges · Natural products · Bacteria · Fungi · Industrial enzymes · Biotechnology

Introduction

Since the 1970s, more than 15,000 structurally diverse natural products with an astounding array of bioactivities have been discovered from the marine environment [13]. Sponges (*Porifera*) are known to be a rich source of bioactive secondary metabolites that are of biotechnological interest because of their antiviral, antitumor, antimicrobial or general cytotoxic properties. Most of these described marine natural compounds originate from sponges, which represent one of the oldest animal phyla [17, 46]. So far about 15,000 species have been described, but the true diversity is probably higher [14, 28]. As filter feeders, sponges pump a large volume (up to 24 m^3 kg^{-1} sponge day^{-1}) of seawater, which contains 1–5 × 10^6 bacteria ml^{-1} [37, 80]. Sponges are well known to be hosts for a large community of microorganisms, such as bacteria and fungi, and some of these microbes are probably host-specific. Microbes can compose up to 50% of the sponge tissue volume [23, 77]. That exceeds the number of bacteria in seawater by two–three orders of magnitude [17, 75, 81]. Through true sponge–microbe symbioses, sponges may benefit from the provision of nutrients, transportation of waste products or active

G. Wang
Department of Oceanography,
University of Hawaii at Manoa,
1680 East-West Road, POST 103B,
Honolulu, HI 96822, USA
E-mail: guangyi@hawaii.edu
Tel.: +1-808-9563744
Fax: +1-808-9569225

metabolites, chemical defense, or contribution to mechanical structure [20, 37, 76, 85, 86, 89–92, 94]; this relationship has elicited considerable interest among researchers investigating marine sponges as a source of natural products. In particular, accumulating evidence has shown that the bacteria associated with sponges are the origin of many compounds of interest [53, 73]. Recent studies of microbial communities associated with sponges have resulted in identification of new marine microbes, novel natural compounds, and host specificity [1, 52, 71, 73, 84].

Diversity of microbial consortia associated with sponges

Microbial symbiosis is an ubiquitous aspect of life and plays a major part in the emergence and evolution of eukaryotes [64]. Intimate associations between microbes and eukaryotic hosts are common in the marine environment [5, 56, 57]; in particular, marine animals and plants are well known to have developed highly specific relationships with numerous microbes [37]. In this section, we focus on sponge microbial diversity.

Bacteria associated with sponges

The unique nature of the sponge–microbe association, together with the ecological and biotechnological importance of sponges, makes marine sponges an ideal system for the study of microbial diversity, evolution of the marine eukaryote–microbe association, and microbial dispersal associated with bioinvasions [7, 8, 18, 50, 72]. The bacterial load in sponges seems proportionally correlated with the irrigation status of the sponge. Sponges with a poor irrigation system contain high bacterial numbers while the well-irrigated sponges have fewer bacteria within their tissues [78, 85]. Bacterial distribution follows a general pattern within every sponge. Photosynthetically active microorganisms, such as cyanobacteria and eukaryotic algae, are located in the outer, light-exposed tissue layers [61, 90]. Heterotrophic and possibly also autotrophic bacteria populate the inner core [23].

The presence of large numbers of bacteria within marine sponges was first established by microscopic studies [78, 87]. Early studies determined the association of bacteria with sponges based on bacterial morphology and recognized three types of associations of bacteria with sponges [78, 85–87]. The first group contained bacteria similar to those of ambient seawater and not specific to sponges, the second group contained intracellular bacteria that are specific to sponges, and the third group contained bacteria within the sponge mesophyl, also specific to sponges. The characteristic features of the bacterial cell wall (gram-positive or gram-negative) and internal membrane structures also helped to identify several bacterial groups using transmission electron microscopy. Cyanobacteria were noticed in sponges in several studies [24, 66, 89]. The presence of cyanobacteria in sponges suggested the idea of symbiotic microbes in sponges. Indeed, several lines of evidence indicated that some sponges obtain a significant portion of their nutrients from the bacterial symbionts, making the symbiosis a true mutualism. Many shallow-water sponges with cyanobacteria exhibit mechanisms that allow bacterial contribution to the host nutrition through extracellular lysis and phagocytosis [85, 92]. Moreover, symbiotic cyanobacteria have been shown to fix nitrogen to provide their hosts with amino acids and also to control the redox potential within sponge tissue via photosynthesis [89, 94]. On the other hand, cyanobacterial symbionts have been observed to overgrow and kill their host sponge [62]. Different color forms of sponges are attributed to the presence of different cyanobacteria and their production of different secondary metabolites [72]. Heterotrophic bacterial symbionts also contribute to the nutrition of sponges. However, the true symbiotic relationship between heterotrophic bacteria and sponges has only been proved in a few cases [29].

Physiologically and phylogenetically diverse sponge-associated microbes have been identified using cultivation-dependent methods [21, 63, 81, 93]. However, the contribution of cultivation to microbial diversity in sponges is limited by the cultivation processes. The selectivity of culture conditions has been a great concern to researchers because the selected strains can become the dominant population under culture conditions even if they are minor components in the sponge. Nevertheless, bacterial isolates with special physiological properties, such as aerobic chemoheterotrophic bacteria [93], nitrogen-fixing bacteria [89], methane-oxidizing bacteria [79], phototrophic bacteria [68, 89], and anoxygenic phototrophic bacteria [30] have been isolated from marine sponges. Most recently, novel actinobacteria were cultivated from several marine sponges [35, 45]. As in other natural habitats, culturable microbes in sponges represent only a small percentage of the total bacterial community. The percentage of the culturable microbial community varies among sponge species and represents 3–11, 0.1–0.23, and 0.15% of the total bacterial population of *Ceratoporella nicholsoni*, *Rhopaloides odorabile*, and *Aplysina aerophoba*, respectively [17, 63, 81]. These observations are generally consistent with an estimated 1% of culturable microbes in the context of natural microbial ecosystems [23].

In the last decade, the novel applications of several molecular cultivation-independent techniques, including 16S rDNA sequencing, denaturing gradient gel electrophoresis (DGGE), and fluorescence in situ hybridization (FISH), have resulted in a remarkable array of new discoveries on marine microbial communities in sponges (for recent reviews, see [23, 24, 25, 29, 38, 50]). These discoveries have greatly improved our understanding of the microbial complexity of the sponge-associated microbes and facilitated the detection of specific groups of prokaryotes without cultivation. A wider range of sponge-associated microbes have been described because of the development of the new detection techniques. These include a diverse range of heterotrophic bacteria, cyanobacteria, facultative anaerobes, unicellular algae, and archaea [16, 17, 21–23, 39, 40, 57, 66, 67, 82–84]. In addition, planktonic and benthic diatoms have also been reported living inside Antarctic sponges [9]. Generally, the phylogenetic signature of the sponge-associated microbial consortium is distinctly different from that of open seawater [19, 22]. Molecular analyses support that bacterial communities are consistently associated with a particular species [84]. Some of the sponge-associated bacteria could be passed on from generation to generation [74, 88]. Substantial variability exists in microbial communities from different sponge species, and therefore, some bacteria are thought to be host-specific "specialists" [71]. Most recently, a new group of bacteria, called "Poribacteria," has been identified in several sponges and proved to be sponge-specific using molecular approaches [14]. Phylogenetic analyses of the isolates of the cyanobacterium *Oscillatoria spongeliae* from different species of *Dysidea* sponges also reveal a high degree of host specificity [72]. The mitochondrial marker cytochrome oxidase and other host specificity studies support the coevolution between heterotrophic bacteria, cyanobacteria, and their sponge hosts [72, 85, 87, 89]. Sponge–microbe associations could date back to Precambrian times about 500 million years ago [88]. The widely distributed and evolutionarily ancient relationship makes sponges ancient niches for microbes [16, 23, 57, 66]. As living fossils, sponges may contain genetic fingerprints for the origin of their microbes and could be good hosts for study of microbial evolution and biogeography.

Fungi associated with sponges

Marine fungi have long been known to exist in the marine environment. Ecologically, they are important intermediaries of energy flow from detritus to higher trophic levels and play an important role in nutrient regeneration cycles as decomposers of dead and decaying organic matter. Moreover, some marine fungi also cause diseases of marine animals and plants while others form mutualistic symbiotic relationships with other organisms. Some marine fungi produce toxins, and others are pathogens to both immune-compromised marine animals and beach-swimmers. However, marine fungi remain the most underexplored group in the marine environment. It is particularly true for fungi associated with sponges. Sponges exhibit an ubiquitous association with diverse marine fungi [47, 72]. Research reports on sponge-derived fungi typically have concentrated on natural product chemistry, studies dealing with biology of fungi in sponges are rare [6, 27]. Marine ascomycetes of the genus *Koralionastes* was reported to have a unique association with crustaceous sponges, developing their ascomata on or within these hosts [36]. In a search for new natural compounds from fungi isolated from sponges collected from six different locations, including temperate, subtropical, and tropical regions, a total 681 fungal strains were isolated from 16 species of sponges [27]. The isolated fungi belonged to 13 genera of Ascomycota, 2 of Zygomycota, and 37 of mitosporic fungi. The diversity of genera and the number of isolates per sample varied greatly between the different locations. *Acremonium*, *Arthrium* sp., *Coniothyrium*, *Fusarium*, *Mucor*, *Penicillium*, *Phoma*, *Trichoderma*, and *Verticilum* sp. strains were isolated from almost all locations although different fungal genera were prevalent at the various locations. In addition, certain fungal genera predominated in some sponges, and the prevalent fungal genera varied in sponges sampled from the same location. However, identification of fungal isolates was solely based on morphology [27]. In another study of distribution of marine filamentous fungi associated with marine sponges in Coral reefs of Palau and Bunaken Island (Indonesia), morphological characters used for identification revealed that the number of fungi associated with sponges sampled at different sites varied greatly between collection sites [47]. Unfortunately, the lack of information, such as the generic label of fungal isolates and the names of sponges, made it difficult to judge diversity and distribution of fungi associated with the marine sponges in this study. Phylogenetic analyses based on ITS sequences indicated that the diversity of fungal culturable population varied among the Hawaiian sponges *Suberites zeteki* and *Mycale armata* (Li and Wang, unpublished data). In general, several lines of evidence support the idea that fungi are ubiquitously associated with sponges, but the nature of the association is unknown [6, 27].

Biotechnological potential of sponge-associated microbes

Sponges are well known to produce a number of biologically active secondary metabolites, which are used as repellents against predators and also manage the sponge-associated microbes and microfauna [12, 58]. Among marine invertebrates, sponges remain the most prolifically studied phylum in the search for novel pharmacologically active compounds [11]. Some enzymes with interesting features have been isolated from sponges and the microbes associated with sponges [38, 39]. In the last two decades, tremendous progress has been made in the isolation and chemistry of secondary metabolites from bacteria and fungi associated with [6, 33, 53, 55, 60]. In this section, we briefly review the status of pharmaceutical compounds and enzymes from microbes associated with sponges.

Pharmaceutical compounds from sponge-associated microbes

Indeed, many natural products with novel bioactivities have been discovered from sponges [59, 60]. These compounds range from derivatives of amino acids and nucleotides to macrolides, porphyrins, terpenoids, and sterols with diverse bioactivities [72]. Many sponge-derived metabolites have been suspected to be actually produced by microbes, since they resemble microbial natural products [12, 53]. Several studies have demonstrated that microbial isolates associated with sponges produced the same compounds isolated from sponges [3, 15, 69, 76]. However, these results do not rule out the possibility that substances might be transported between different cell types via export or sequestration mechanisms [55]. Recent studies of the culturable sponge microbial communities have resulted in the identification of new natural compounds with diverse biological activities [15, 21, 26, 73, 76]. Detailed information on natural compounds from bacteria isolated from sponges was recently reviewed [53]. Hence, sponges have been considered as "microbiological fermentors" for new natural products with potential biotechnological applications [72]. Furthermore, metagenomic approaches have revealed novel chemistry of the uncultivated symbionts in marine sponge *Theonella swinhoei* and *Discodermia dissoluta* and have opened a new avenue for a better understanding of the valuable natural compounds from symbiotic bacteria in sponges [54, 55, 65]. Due to the bias of DNA extraction and cloning during library construction, much more work is needed to address the reliability of biochemical and genetic diversity revealed using metagenomic sponge libraries [65].

Terrestrial fungi produce a variety of chemically diverse natural compounds with interesting biological activities. Until recently, only modest attention has been devoted toward metabolites from marine (or marine-derived) fungi. To date, about 300 new compounds from marine-derived fungi have been described and reviewed [4, 6, 12, 33, 60]. These documented discoveries clearly show that marine fungi are a prolific source of structurally unique and biologically active natural compounds. Fungi derived from sponges, algae, sea grasses, plants, or wood substrates account for the majority of chemistry described for marine fungal natural compounds [6]. Fungi associated with sponges are the single most prolific source of new marine fungal compounds and display diverse biological activities [6, 32]. These fungi account for the largest number (33%) of total described marine fungal compounds and produce the overall highest number of novel metabolites (28%) [6]. However, a high percentage of marine fungi derived from sponges is considered to be of terrestrial origin, instead of marine origin [27]. The current isolation methods select for the fastest growing strains with no selection on dominant strains in sponges [32]. Hence, morphology-based taxonomy for sponge marine strains needs to be reevaluated using molecular approaches. Fermentation conditions (e.g., salt concentration) appear to significantly affect the profiles of secondary metabolites from marine strains, but different species behave differently in the same conditions [6, 31, 48, 49, 70]. The marine-derived fungi *Phoma* sp. and *Aspergillus sydowii* differ significantly from their terrestrial counterparts in secondary metabolite profiles [2, 51]. Therefore, studies of metabolic pathways of both a sponge marine fungus and its terrestrial counterpart may reveal their differences at a molecular level.

Enzymes from sponge-associated microbes

The microhabitats on the surface and internal spaces of sponges are unique ecological niches. As filter feeders, sponges are exposed to pollutants present in waters, and accumulated impurities from phytoplankton, or other suspended matters. Sponges swirl in a large volume of seawater containing organic particles. Hence, it is reasonable to believe that some microbes in sponges and/or sponges themselves produce hydrolytic enzymes to convert these organic matters into nutrients. In contrast to the literature on natural compounds, studies on enzymes with biotechnological potential from microbes associated with sponges are rare. Amylases, carboxymethylcellulases, proteases, and other hydrolytic enzymes are widely used in the industry for the manufacture of pharmaceuticals, foods, beverages, and confectionery as well as in textile and leather processing, and waste water treatment [95]. Species of the bacterial genus *Cytophaga* can hydrolyze agar and were identified from the sponge *Halichondria panacea* [29]. Several *Desulfovibrio* bacteria with ability to dehalogenate and degrade brominated compounds were isolated from the marine sponge *A. aerophoba* [1]. Bacteria isolated from six marine sponges (*Spirastrella* sp., *Phyllospongia* sp., *Ircinia* sp., *Aaptos* sp., *Azorica* sp., and *Axinella* sp.) were identified to produce amylase, carboxymethylcellulase, and proteases [44]. Furthermore, the bacterium *Arthrobacter ilicis* and fungus *Mucor* sp. isolated from the sponge *Spirastrella* sp. were found to produce an acetylcholinesterase and amylase, respectively [42, 43]. These two enzymes were heat-tolerant and their activities not affected by the major cations of seawater, such as Na^+, Ca^{2+}, and Mg^{2+}, at relatively high concentrations. The metagenomic approach has also identified several novel enzymes with potential application in environmental biotechnology, including a molybdenum containing oxidoreductase and halogenases from marine sponges (U. Hentschel group, personal communication). Finally, the bacterium *Micrococcus* sp. associated with the *Spirastrella* sponge produced urethanase, which could potentially be used to remove urethane, a cancer-causing chemical, from alcoholic beverages [41].

Conclusion

Recent advances in understanding sponge–microbe associations have dramatically enriched our knowledge on the diversity, population ecology, and biotechnology of sponge-associated microbes. However, the information on diversity of the sponge-associated microbes is still fragmentary, and many aspects of the sponge–microbe associations are still unexplored. In particular, our understanding of marine microbial diversity in sponges has been focused on bacteria, with much less emphasis on eukaryotes (e.g., fungi) [71]. Exploration of biotechnological potentials of microbes associated with sponges has been limited due to our inability to cultivate these microbes associated with sponges. Several methods to simulate native habitat conditions and to use low-nutrient media have been successful to cultivate new marine bacteria from different marine habitats [10, 34, 96]. Although sponge agar and other media have been used to cultivate microbes from sponges [21, 52, 81], new cultivation methods are clearly needed for better exploration of biotechnological potential of sponge-associated microbes. Environmental genomic approaches could be useful to explore uncultured microbial populations for the purpose of producing new chemical compounds and industrial enzymes. Other microbes, compounds with novel structures and biological activities, and enzymes with desirable industrial features will be identified from microbial consortia associated with sponges in the near future using new microbiological cultivation methods, molecular techniques, and analytical tools. Particularly, coordination among microbiologists, natural product chemists, mycologists, and bioengineers to investigate biodiversity and biotechnological potential of sponge-associated microbes will contribute significantly to the pharmaceutical and enzyme industries.

References

1. Ahn YB, Rhee SK, Fennell DE, Kerkhof LJ, Hentschel U, Haggblom MM (2003) Reductive dehalogenation of brominated phenolic compounds by microorganisms associated with the marine sponge *Aplysina aerophoba*. Appl Environ Microbiol 69:4159–4166
2. Alker AP, Smith GW, Kim K (2001) Characterization of *Aspergillus sydowii* (Thom et Church), a fungal pathogen of Caribbean sea fan corals. Hydrobiologia 460:105–111
3. Bewley CA, Holland ND, Faulkner DJ (1996) Two classes of metabolites from *Theonella swinhoei* are localized in distinct populations of bacterial symbionts. Experientia 52:716–722
4. Biabani MAF, Laatsch H (1998) Advances in chemical studies on low-molecular weight metabolites of marine fungi. J Prakt Chem 340:589–607
5. Brantley SE, Molinski TF, Preston CM, Delong EF (1995) Brominated acetylenic fatty acids from *Xestospongia* sp., a marine sponge–bacteria association. Tetrahedron 51:7667–7672
6. Bugni TS, Ireland CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat Prod Rep 21:143–163
7. Carballo JL, Naranjo S (2002) Environmental assessment of a large industrial marine complex based on a community of benthic filter-feeders. Mar Pollut Bull 44:605–610
8. Carballo JL, Naranjo SA, Garciagomez JC (1996) Use of marine sponges as stress indicators in marine ecosystems at Algeciras Bay (southern Iberian Peninsula). Mar Ecol Prog Ser 135:109–122
9. Cerrano C, Arillo A, Bavestrello G, Calcinai B, Cattaneo-Vietti R, Penna A, Sara M, Totti C (2000) Diatom invasion in the Antarctic hexactinellid sponge *Scolymastra joubini*. Polar Biol 23:441–444
10. Connon SA, Giovannoni SJ (2002) High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl Environ Microbiol 68:3878–3885
11. Faulkner DJ (2000) Marine natural products. Nat Prod Rep 17:7–55
12. Faulkner DJ (2001) Marine natural products. Nat Prod Rep 18:1–49
13. Faulkner DJ, Harper MK, Haygood MG, Salomon CE, Schmidt EW (2000) Symbiotic bacteria in sponges: sources of bioactive substances. In: Fusetani N (ed) Drugs from the sea. Karger, Basel, pp 107–119
14. Fieseler L, Horn M, Wagner M, Hentschel U (2004) Discovery of the novel candidate phylum “Poribacteria” in marine sponges. Appl Environ Microbiol 70:3724–3732
15. Flowers AE, Garson MJ, Webb RI, Dumdei EJ, Charan RD (1998) Cellular origin of chlorinated diketopiperazines in the dictyoceratid sponge *Dysidea herbacea* (Keller). Cell Tissue Res 292:597–607
16. Friedrich AB, Merkert H, Fendert T, Hacker J, Proksch P, Hentschel U (1999) Microbial diversity in the marine sponge *Aplysina cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence in situ hybridization (FISH). Mar Biol 134:461–470
17. Friedrich AB, Fischer I, Proksch P, Hacker J, Hentschel U (2001) Temporal variation of the microbial community associated with the Mediterranean sponge *Aplysina aerophoba*. FEMS Microbiol Ecol 38:105–113
18. Gili JM, Coma R (1998) Benthic suspension feeders—their paramount role in littoral marine food webs. Trends Ecol Evol 13:316–321
19. Giovannoni SG, Rappe MS (2000) Evolution, diversity and molecular ecology of marine prokaryotes. In: Kirchman DL (ed) Microbial ecology of the ocean. Wiley, New York
20. Haygood MG, Schmidt EW, Davidson SK, Faulkner DJ (2000) Microbial symbionts of marine invertebrates: opportunities for microbial biotechnology. In: Bartlett DH (ed) Molecular marine microbiology. Horizon Scientific Press, Wymondham, England, pp 61–84
21. Hentschel U, Schmid M, Wagner M, Fieseler L, Gernert C, Hacker J (2001) Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. FEMS Microbiol Ecol 35:305–312
22. Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440
23. Hentschel U, Fieseler L, Wehrl A, Gernert C, Steinert M, Hacker J, Horn M (2003) Microbial diversity of marine sponges. In: Müller WEG (ed) Sponges (Porifera). Springer, Berlin Heidelberg New York, pp 59–88
24. Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. FEMS Microbiol Ecol 55:167–177
25. Hill RT (2004) Microbes from marine sponges: a treasure trove of biodiversity for natural products discovery. In: Bull AT (ed) Microbial diversity and bioprospecting. ASM Press, Washington, pp 177–190
26. Hinde R, Pironet F, Borowitzka MA (1994) Isolation of *Oscillatoria spongeliae*, the filamentous cyanobacterial symbiont of the marine sponge *Dysidea herbacea*. Mar Biol 119:99–104
27. Holler U, Wright AD, Matthee GF, Konig GM, Draeger S, Aust HJ, Schulz B (2000) Fungi from marine sponges: diversity, biological activity and secondary metabolites. Mycol Res 104:1354–1365
28. Hooper JNA, van Soest RWM (2002) Systema Porifera. A guide to the classification of sponges, vol 1. Plenum Publishers, New York
29. Imhoff JF, Stoehr R (2003) Sponge-associated bacteria: general overview and special aspects of bacteria associated with *Halichondria panicea*. In: Mueller WEG (ed) Sponges (Porifera). Springer, Berlin Heidelberg New York, pp 35–57
30. Imhoff JF, Trueper HG (1976) Marine sponges as habitats of anaerobic phototrophic bacteria. Microb Ecol 3:1–9
31. Iwamoto C, Minoura K, Hagishita S, Nomoto K, Numata A (1998) Penostatins f–i, novel cytotoxic metabolites from a *Penicillium* species separated from an enteromorpha marine alga. J Chem Soc Perkin Trans 1:449–456

32. Jensen PR, Fenical W (2000) Marine microorganisms and drug discovery: current status and future potential. Drugs from the sea, pp 6–29
33. Jensen PR, Fenical W (2002) Secondary metabolites from marine fungi. In: Kevin DH (ed) Fungi in marine environments. Fungal Diversity Press, Hong Kong, pp 293–315
34. Kaeberlein T, Lewis K, Epstein SS (2002) Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. Science 296:1127–1129
35. Kim TK, Garson MJ, Fuerst JA (2005) Marine actinomycetes related to the '*Salinospora*' group from the Great Barrier Reef sponge *Pseudoceratina clavata*. Environ Microbiol 7:509–518
36. Kohlmeyer J, Volkmann-Kohlmeyer B (1991) Illustrated key to the filamentous higher marine fungi. Bot Mar 34:1–35
37. Kubanek J, Jensen PR, Keifer PA, Sullards MC, Collins DO, Fenical W (2003) Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi. Proc Natl Acad Sci USA 100:6916–6921
38. Lee YK, Lee J-H, Lee HK (2001) Microbial symbiosis in marine sponges. J Microbiol 39:254–264
39. Manz W, Arp G, Schumann-Kindel G, Szewzyk U, Reitner J (2000) Widefield deconvolution epifluorescence microscopy combined with fluorescence in situ hybridization reveals the spatial arrangement of bacteria in sponge tissue. J Microbiol Methods 40:125–134
40. Margot H, Acebal C, Toril E, Amils R, Puentes JLF (2002) Consistent association of crenarchaeal archaea with sponges of the genus *Axinella*. Mar Biol 140:739–745
41. Mohapatra BR, Bapuji M (1997) Characterization of urethanase from *Micrococcus* species associated with the marine sponge (*Spirastrella* species). Lett Appl Microbiol 25:393–396
42. Mohapatra BR, Bapuji M (1998) Characterization of acetylcholinesterase from *Arthrobacter ilicis* associated with the marine sponge (*Spirastrella* sp.). J Appl Microbiol 84:393–398
43. Mohapatra BR, Banerjee UC, Bapuji M (1998) Characterization of a fungal amylase from *Mucor* sp. associated with the marine sponge *Spirastrella* sp. J Biotechnol 60:113–117
44. Mohapatra BR, Bapuji M, Sree A (2003) Production of industrial enzymes (amylase, carboxymethylcellulase and protease) by bacteria isolated from marine sedentary organisms. Acta Biotechnol 23:75–84
45. Montalvo NF, Mohamed NM, Enticknap JJ, Hill RT (2005) Novel actinobacteria from marine sponges. Antonie Van Leeuwenhoek 87:29–36
46. Müller WEG, Müller IM (2003) Analysis of the sponge (Porifera) gene repertoire: implications for the evolution of the metazoan body plan. In: Müller WEG (ed) Sponges (Porifera). Springer, Berlin Heidelberg New York, pp 1–33
47. Namikoshi M, Akano K, Kobayashi H, Koike Y, Kitazawa A, Rondonuwu AB, Pratasik SB (2002) Distribution of marine filamentous fungi associated with marine sponges in coral reefs of Palau and Bunaken Island, Indonesia. J Tokyo Univ Fish, pp 15–20
48. Numata A, Takahashi C, Ito Y, Takada T, Kawai K, Usami Y, Matsumura E, Imachi M, Ito T, Hasegawa T (1993) Communesins, cytotoxic metabolites of a fungus isolated from a marine alga. Tetrahedron Lett 34:2355–2358
49. Numata A, Takahashi C, Ito Y, Minoura K, Yamada T, Matsuda C, Nomoto K (1996) Penochalasins, a novel class of cytotoxic cytochalasans from a penicillium species separated from a marine alga—structure determination and solution conformation. J Chem Soc Perkin Trans 1:239–245
50. Osinga R, Armstrong E, Burgess JG, Hoffmann F, Reitner J, Schumann-Kindel G (2001) Sponge–microbe associations and their importance for sponge bioprocess engineering. Hydrobiologia 461:55–62
51. Osterhage C, Schwibbe M, Konig GM, Wright AD (2000) Differences between marine and terrestrial *Phoma* species as determined by HPLC-DAD and HPLC-MS. Phytochem Anal 11:288–294
52. Pabel CT, Vater J, Wilde C, Franke P, Hofemeister J, Adler B, Bringmann G, Hacker J, Hentschel U (2003) Antimicrobial activities and matrix-assisted laser desorption/ionization mass spectrometry of Bacillus isolates from the marine sponge *Aplysina aerophoba*. Mar Biotechnol 5:424–434
53. Piel J (2004) Metabolites from symbiotic bacteria. Nat Prod Rep 21:519–538
54. Piel J, Hui D, Wen G, Butzke D, Platzer M, Fusetani N, Matsunaga S (2004) Antitumor polyketide biosynthesis by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei*. Proc Natl Acad Sci USA 101:16222–16227
55. Piel J, Butzke D, Fusetani N, Hui D, Platzer M, Wen G, Matsunaga S (2005) Exploring the chemistry of uncultivated bacterial symbionts: antitumor polyketides of the pederin family. J Nat Prod 68:472–479
56. Preston CM, Delong EF (1995) Sponge-associated marine crenarchaeota: evidence for an archaeal-metazoan symbiosis. Abstr Gen Meet ASM 95:327
57. Preston CM, Wu KY, Molinski TF, Delong EF (1996) A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen., sp. nov. Proc Natl Acad Sci USA 93:6241–6246
58. Proksch P (1994) Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs. Toxicon 32:639–655
59. Proksch P, Edrada RA, Ebel R (2002) Drugs from the seas—current status and microbiological implications. Appl Microbiol Biotechnol 59:125–134
60. Proksch P, Ebel R, Edrada RA, Wray V, Steube K (2003) Bioactive natural products from marine invertebrates and associated fungi. In: Müller WEG (ed) Sponges (Porifera). Springer, Berlin Heidelberg New York, pp 117–138
61. Rutzler K (1985) Associations between Caribbean sponges and photosynthetic organisms. In: Rutzler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, pp 455–466
62. Rutzler K (1988) Mangrove sponge disease induced by cyanobacterial symbionts failure of a primitive immune system. Dis Aquat Organ 5:143–150
63. Santavy DL, Willenz P, Colwell RR (1990) Phenotypic study of bacteria associated with the Caribbean sclerosponge *Ceratoporella nicholsoni*. Appl Environ Microbiol 56:1750–1762
64. Sapp J (2004) The dynamics of symbiosis: an historical overview. Can J Bot 82:1046–1056
65. Schirmer A, Gadkari R, Reeves CD, Ibrahim F, DeLong EF, Hutchinson CR (2005) Metagenomic analysis reveals diverse polyketide synthase gene clusters in microorganisms associated with the marine sponge *Discodermia dissoluta*. Appl Environ Microbiol 71:4840–4849
66. Schmidt EW, Obraztsova AY, Davidson SK, Faulkner DJ, Haygood MG (2000) Identification of the antifungal peptide-containing symbiont of the marine sponge *Theonella swinhoei* as a novel delta-proteobacterium, "*Candidatus Entotheonella palauensis*". Mar Biol 136:969–977
67. Schumannkindel G, Bergbauer M, Manz W, Szewzyk U, Reitner J (1997) Aerobic and anaerobic microorganisms in modern sponges—a possible relationship to fossilization-processes. Facies 36:268–272
68. Simpson TL (1984) The cell biology of sponges. Springer, Berlin Heidelberg New York
69. Stierle AC, Cardellina JH, Singleton FL (1988) A marine micrococcus produces metabolites ascribed to the Sponge *Tedania ignis*. Experientia 44:1021
70. Takahashi C, Numata A, Yamada T, Minoura K, Enomoto S, Konishi K, Nakai M, Matsuda C, Nomoto K (1996) Penostatins, novel cytotoxic metabolites from a *Penicillium* species separated from a green alga. Tetrahedron Lett 37:655–658
71. Taylor MW, Schupp PJ, Dahllof I, Kjelleberg S, Steinberg PD (2004) Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. Environ Microbiol 6:121–130
72. Thakur NL, Muller WEG (2004) Biotechnological potential of marine sponges. Curr Sci 86:1506–1512
73. Thiel V, Imhoff JF (2003) Phylogenetic identification of bacteria with antimicrobial activities isolated from Mediterranean sponges. Biomol Eng 20:421–423

74. Thiel V, Blumenberg M, Hefter J, Pape T, Pomponi S, Reed J, Reitner J, Worheide G, Michaelis W (2002) A chemical view of the most ancient metazoa—biomarker chemotaxonomy of hexactinellid sponges. Naturwissenschaften 89:60–66
75. Thoms C, Horn M, Wagner M, Hentschel U, Proksch P (2003) Monitoring microbial diversity and natural product profiles of the sponge *Aplysina cavernicola* following transplantation. Mar Biol 142:685–692
76. Unson MD, Holland ND, Faulkner DJ (1994) A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. Mar Biol 119:1–11
77. Usher KM, Fromont J, Sutton DC, Toze S (2004) The biogeography and phylogeny of unicellular cyanobacterial symbionts in sponges from Australia and the Mediterranean. Microb Ecol 48:167–177
78. Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. J Exp Mar Biol Ecol 30:301–314
79. Vacelet J, Fialamedioni A, Fisher CR, Bouryesnault N (1996) Symbiosis between methane-oxidizing bacteria and a deep-sea carnivorous cladorhizid sponge. Mar Ecol Prog Ser 145:77–85
80. Vogel S (1977) Current induced flow through living sponges in nature. Proc Natl Acad Sci USA 74:2069–2071
81. Webster NS, Hill RT (2001) The culturable microbial community of the Great Barrier Reef sponge *Rhopaloeides odorabile* is dominated by an *alpha-Proteobacterium*. Mar Biol 138:843–851
82. Webster NS, Watts JEM, Hill RT (2001) Detection and phylogenetic analysis of novel crenarchaeote and euryarchaeote 16S ribosomal RNA gene sequences from a Great Barrier Reef sponge. Mar Biotechnol 3:600–608
83. Webster NS, Webb RI, Ridd MJ, Hill RT, Negri AP (2001) The effects of copper on the microbial community of a coral reef sponge. Environ Microbiol 3:19–31
84. Webster NS, Negri AP, Munro M, Battershill CN (2004) Diverse microbial communities inhabit Antarctic sponges. Environ Microbiol 6:288–300
85. Wilkinson CR (1978) Microbial associations in sponges. Part 1. Ecology physiology and microbial populations of Coral Reef Sponges. Mar Biol 49:161–168
86. Wilkinson CR (1978) Microbial associations in sponges. Part 2. Numerical analysis of sponge and water bacterial populations. Mar Biol 49:169–176
87. Wilkinson CR (1978) Microbial associations in sponges. Part 3. Ultrastructure of the in situ associations in Coral Reef Sponges. Mar Biol 49:177–185
88. Wilkinson CR (1984) Immunological evidence for the Precambrian origin of bacterial symbioses in marine sponges. Proc R Soc Lond 220:509–517
89. Wilkinson CR (1987) Significance of microbial symbionts in sponge evolution and ecology. Symbiosis 4:135–146
90. Wilkinson CR (1992) Symbiotic interactions between marine sponges and algae. In: Reisser W (ed) Algae and symbioses: plants, animals, fungi, viruses, interactions explored. Biopress, Bristol, pp 113–151
91. Wilkinson CR, Fay P (1979) Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. Nature 279:527–529
92. Wilkinson CR, Garrone R (1980) Nutrition of marine sponges: involvement of symbiotic bacteria in the uptake of dissolved carbon. In: Smith DC, Tiffon Y (eds) Nutrition of lower metazoa. Pergamon Press, Oxford, pp 157–161
93. Wilkinson CR, Nowak M, Austin B, Colwell RR (1981) Specificity of bacterial symbionts in Mediterranean and Great Barrier Reef Australia Sponges. Microb Ecol 7:13–22
94. Wilkinson CR, Summons R, Evans E (1999) Nitrogen fixation in symbiotic marine sponges: ecological significance and difficulties in detection. Mem Old Mus 44:667–673
95. Wiseman A (1985) Handbook of enzyme biotechnology. Ellis Horwood, New York
96. Zengler K, Toledo G, Rappe M, Elkins J, Mathur EJ, Short JM, Keller M (2002) Cultivating the uncultured. Proc Natl Acad Sci USA 99:15681–15686