The Journal of Microbiology, December 2001, p.254-264 Vol. 39, No. 4

MINIREVIEW

Microbial Symbiosis in Marine Sponges

Yoo Kyung Lee, Jung-Hyun Lee, and Hong Kum Lee*

Microbiology Laboratory, Korea Ocean Research & Development Institute, Ansan P.O. Box 29, Seoul 425-600, Korea

(Received September 27, 2001 / Accepted November 21, 2001)

Sponges are host organisms for various symbiotic microorganisms such as archaea, bacteria, cyanobacteria and microalgae. Sponges are also sources of a wide variety of useful natural products like cytotoxins, antifouling agents, antibiotics, and anti-inflammatory and antiviral compounds. Symbiotic microorganisms in sponges can be sources of various natural products, because metabolites previously ascribed to sponges have recently been demonstrated to be biosynthesized by symbionts. If a symbiotic microorganism from which some natural products are derived can be cultured, the microorganism could be used in a mass production of the bioactive compounds. We summarize recent research on isolation and cultivation of sponge-symbiotic microorganisms and the symbiotic relationship.

Key words: bioactive compound, natural products, symbiotic microorganism, sponges, symbiosis

Why sponge-symbiotic microorganisms?

Sponges (Phylum Porifera) are very fertile host animals for diverse symbiotic microorganisms. Sponges are simple multicellular invertebrates attached to solid substrates in benthic habitats. All sponges are filter feeders; numerous tiny pores on the surface allow water to enter and circulate through a series of canals where microorganisms and organic particles are filtered out and eaten. Since sponges are efficient filter feeders, any microorganisms that can resist the sponge digestive process and immune response can successfully inhabit sponges (126).

Sponges have been excellent sources for natural products that are bioactive compounds (Table 1). Their bioactivity includes enzyme inhibitors, cell division-inhibitors, antiviral, antifungal, antimicrobial, anti-inflammatory, antitumor, cytotoxic or cardiovascular properties (79). Reports of the isolation of natural products from marine sponges have been published from the early 1950's (4), and research activities on this topic have continued to increase (79). Nowadays, several papers on new natural products from sponges are published monthly; and Faulkner (27) has published surveys on many more natural products recently isolated from sponges.

Many of these natural products have interesting biomedical potential, pharmaceutical relevance and diverse applications (44, 46). For example, arabinose-nucleosides with antiviral and anticancer activity isolated from sponge *Cryptotethya crypta*, are used clinically; manoalide obtained from sponge *Luffariella variabilis* is a candidate for new drugs with anti-inflammatory activity (68). Also, metabolites previously ascribed to sponges have been recently demonstrated to be biosynthesized by symbionts. If some compounds are derived from a symbiotic microorganism, culturing the microorganism could provide an improved source of the bioactive compound (97). Thus, we have focused on sponge-symbiotic microorganisms as a source of various natural products in this review.

* To whom correspondence should be addressed.
(Tel) 82-31-400-6241; (Fax) 82-31-406-2495
(E-mail) hklee@kordi.re.kr

What microorganisms live in sponges?

Various microorganisms have been found in sponges (Table 2). They include a diverse range of archaea, heterotrophic bacteria, cyanobacteria, green algae, red algae, cryptophytes, dinoflagellates and diatoms (22, 70, 88, 96). The symbiotic microbial community is highly novel and diverse, and species composition shows temporal and geographic variation (121). However, only very little information exists about the taxonomic affiliation of sponge-symbiotic microorganisms (32).

One host sponge can possess diverse symbionts. For example, sponge *Theonella swinhoei* supports unicellular heterotrophic bacteria, unicellular cyanobacteria and filamentous heterotrophic bacteria at the same time (7). A sponge belonging to *Aplysina* includes heterogeneous bacteria *Bacillus* sp., *Micrococcus* sp., *Arthrobacter* sp., *Vibrio* sp., *Pseudoalteromonas* sp., and so on (46). Sponge *Rho-*

Table 1. Sponges and their natural products showing various bioactivity

Sponge	Bioactive metabolites	Biological activity	Reference
Acanthella sp.	Kalihinol-A	Antibiotic	16
Agelas dispar	Aminozooanemonin	Antibacterial	11
Agelas dispar	Pyridinebetaine A	Antibacterial	11
Agelas mauritiana	Agelasimine	Cytotoxic	25
Agelas mauritiana	Sceptrin	Antimicrobial	5
Agelas nakamurai	Ageliferine	Antibacterial	23
Agelas nakamurai	Debromosceptrin	Antibacterial	23
Agelas nakamurai	Nakamuric acid	Antibacterial	23
Agelas novaecaledoniae	Ageliferine	Somatostatin/VIP inhibitor	119
Agelas novaecaledoniae	Sceptrin	Somatostatin/VIP inhibitor	119
Agelas novaecaledoniae	Xestospongine B	Somatostatin/VIP inhibitor	119
Agelas sp.	Agelasine	Antileukemic	53
Agelas sp.	Agelasine F	Antituberculosis	72
Agelas sp.	Agelasine I	Antimicrobial	33
Amphimedon sp.	Pyrinodemin	Cytotoxic	112
Aplysina aerophoba	Aeroplysinin I	Cytotoxic	69
Batzella sp.	Discorhabdin	Cytotoxic, enzyme inhibitor	42
Batzella sp.	Secobatzelline	Phosphatase inhibitor	41
Crella sp.	Crellastatins	Cytotoxic	38
Corticium sp.	Meridine	Antifungal	75
Cymbastela sp.	Agelastatins C, D	Insecticidal	50
Discodermia calyx	Calyculin A	Antitumor	59
Discodermia kiiensis	Discodermin A	Antimicrobial	73
Discodermia sp.	Discobahamins	Antifungal	43
Disidea avara	Avarol	Cytotoxic	77
Druinella purpurea	Psammaplysin C	Cytotoxic	18
Dysidea sp.	Furodysinin	Antiparasitic	78
Echinoclathria sp.	Echinoclathrines	Immunosuppressive	62
Erylus lendenfeldi	Eryloside A	Antitumor, antifungal	13
Fascaplysinopsis reticulata	β-Carbolium salt	Antiparasitic	55
Halichondria okadai	Halichondrin B	Antitumor	48
Haliclona osiris	Osirisynes	Na^+/K^+-ATPase inhibitor	104
Haliclona sp.	Manzamine A	Antitumor	94
Haliclona tulearensis	Halitulin	Cytotoxic	58
Hamacantha sp.	Hamacanthin	Antifungal	40
Hyrtios erecta	Heteronemin	Antiparasitic	66
Ianthella basta	Bastadin	Antimicrobial	87
Ianthella sp.	34-sulfatobastadin 13	Endothelin A receptor inhibitor	39
Ircinia sp.	Haterumalides	Cytotoxic	109
Jaspis johnstoni	Jasplakinolide	Cytotoxic	8
Jaspis johnstoni	Jasplakinolide	Insecticidal	19
Jaspis johnstoni	Toyocamycin	Cytotoxic	130
Jaspis johnstoni	Tubercidin	Cytotoxic	130
Jaspis sp.	Bengamides	Antitumor	111
Jaspis sp.	Bengazoles	Antiparasitic	92
Jaspis sp.	Cyclodepsipeptide	Antifungal	101
Jaspis sp.	Jaspisamides	Cytotoxic	64
Jaspis sp.	Psammaplin	Antibacterial	61
Jaspis splendans	Jaspamide	Antitumor	131
Jaspis wondoensis	Wondosterols	Antimicrobial	93
Latrunculia magnifica	Latrunculin A	Neurotoxin	57
Leucetta cf. chagosensis	Isonaamidine D	Antifungal	34
Neosiphonia superstes	Sphinxolides	Cytotoxic	12
Notodoris citrina-Leucetta chagosensis	Naamidines & naamines	Antiparasitic	13

Table 1. Continued

Sponge	Bioactive metabolites	Biological activity	Reference
Pachastrissa sp.	Bengamides	Antifungal	29
Pachastrissa sp.	Bengazoles	Antifungal	29
Pandaros acanthifolium	Acanthifolicin	Antitumor	99
Petrosia sp.	Petrocortynes	Cytotoxic, enzyme inhibitor	105
Petrosia sp.	Petrotetrayndiols	Cytotoxic	60
Petrosia sp.	Petrosiacetylenes	Na^+/K^+-ATPase inhibitor	102
Plakinastrella sp.	Elenic acid	Topoisomerase II ihibitor	56
Poecillastra wondoensis	Wondosterols	Antimicrobial	93
Psammaplysilla crassa	Purealin	Antiparasitic	81
Psammaplysilla purpurea	Aeroplysinin I	Antiparasitic	71
Psammaplysilla purpurea	Bastadin	Antimicrobial	14
Psammaplysilla purpurea	Purealidin A	Cytotoxic	52
Reidispongia coerulea	Reidispongiolide	Cytotoxic	12
Reniera cratera	Dorimidazole A	Antiparasitic	17
Rhaphisia lacazei	Topsentins	Antiproliferative	15
Spongia sp.	Spongianolide	Cytotoxic	45
Spongionella gracilis	Gracilin B	Cytotoxic	74
Stronglyophora hartmani	Puupehenone	Cytotoxic	67
Stylinos n. sp.	Mycalamides	Cytotoxic	106
Suberea creba	Aeroplysinin I	Antibacterial	21
Suberea creba	Dibromoverongiaquinol	Antibacterial	21
Tedania digitata	1-methylisoguanosine	Cardiovascular effector	91
Tedania ignis	Tedanolide	Cytotoxic	100
Tethya crypta	Spongouridine, Spongothymidine	Antiviral, antitumor	4
Theonella sp.	Koshikamide	Cytotoxic	35
Theonella swinhoei	Swinholide	Antifungal	65
Theonella swinhoei	Theopederins	Antifungal, cytotoxic	113
Verongia aerophoba	Aeroplysinin I	Antibacterial	26
Verongia aerophoba	Dienone	Cytotoxic	110
Verongia spengelii	Aplysinopsin	Cytotoxic	49
Xestospongia sp.	Xestospongine B	Somatostatin/VIP inhibitor	119
Xestospongia sp.	Agcliferine	Somatostatin/VIP inhibitor	119
Xestospongia sp.	Sceptrin	Somatostatin/VIP inhibitor	119
Xestospongia sp.	Xestoaminol A	Antiparasitic	55
Zyzzya fuliginosa	Veiutamine	Cytotoxic	120

paloeides odorabile has β-*Proteobacteria*, γ-*Proteobacteria*, *Cytophaga*, *Actinobacteria* and green sulfur bacteria (122).

Does species-specificity exist in the sponge-microorganism relationship? Some symbionts seem to inhabit specific sponges, but others don't. Wilkinson (129) found that a symbiotic microorganism was specific to a single sponge species. A species of δ-*proteobacteria* and the sponge *Theonella swinhoei* also show a specific association (97). Some sponges have a dominant symbiotic microorganism. A species of α-*proteobacteria* dominates in sponge *Rhopaloeides odorabile* over various habitats but is not detected from seawater, which strongly suggests that the symbiont is species specific (121). The marine sponge *Halichondria panicea* harbors a dominant bacterial species belonging to the genus *Rhodobacter* (1). On the other hand, one symbiont occurs commonly in various sponges from different regions, so seems to possess a wide host range (129). For example, cyanobacteria *Aphanocapsa* sp., *Phormidium* sp., or *Oscillatoria spongeliae* are found in numerous sponges (125, 128).

Where do microorganisms live in sponges?

The symbionts locate both intra- and extra cellularly (Fig. 1), and each symbiotic microorganism seems to have a specific habitat in the host sponge. Extracellular symbionts are present on the outer layers of sponges as exosymbionts (Fig. 1A; 44, 108), or in the mesohyl as endosymbionts (Fig. 1B; 1, 30, 47, 97). Intracellular or intranuclear symbionts permanently reside in host cells or nuclei (Fig. 1C, 1D; 30, 32, 117). In the case of the sponge *Theonella swinhoei*, all populations of symbiotic bacteria are located extracellularly (7). Bacteria, e.g., *Pseudomonas* sp. and *Aeromonas* sp. inhabit as free-living cells in the mesohyl

Table 2. Sponges and their symbiotic microorganisms producing natural products

Sponge	Symbiotic microorganism[a]	Natural product[b]	Reference
Aciculites orientalis	Filamentous bacteria	Theonegramide	7
Antarctic sponge	B, *Pseudomonas aeruginosa*	NC	54
Aplysina sp.	B, *Arthrobacter* sp.	NC	46
Aplysina sp.	B, *Bacillus* sp.	NC	46
Aplysina sp.	B, *Micrococcus* sp.	NC	46
Aplysina sp.	B, *Pseudoalteromonas* sp.	NC	46
Aplysina sp.	B, *Vibrio* sp.	NC	46
Cenarchaeum symbiosum	Archeon	NC	88
Dysidea herbacea	Cyanobacterium	Chlorinated metabolites	115
Dysidea herbacea	C, *Oscillatoria spongeliae*	Polybrominated biphenyl ethers	30
Dysidea sp.	B, *Vibrio* sp.	Brominated biphenyl ethers	24
Halichondria okadai	B, *Alteromonas* sp.	Alteramide A	103
Halichondria okadai	D, *Prorocentrum lima*	Okadaic acid	63
Halichondria panicea	B, *Antarcticum vesiculatum*	Neuroactive compounds	86
Halichondria panicea	B, *Pseudomonas insolita*	NC	80
Halichondria panicea	B, *Rhodobacter* sp.	NC	1
Halichondria panicea	B, *Psychroserpens burtonensis*	Neuroactive compounds	86
Homophymia sp.	B, *Pseudomonas* sp.	Antimicrobial compounds	10
Hyatella sp.	B, *Vibrio* sp.	NC	82
Rhopaloeides odorabile	B, β-*Proteobacteria*	NC	122
Rhopaloeides odorabile	B, γ-*Proteobacteria*	NC	122
Rhopaloeides odorabile	A, *Actinobacteria* sp.	NC	122
Rhopaloeides odorabile	B, *Cytophaga* sp.	NC	122
Rhopaloeides odorabile	Green sulfur bacteria	NC	122
Sigmadocia symbiotica	R, *Ceratodictyon spongiosum*	NC	89
Suberea creba	B, *Pseudomonas* sp.	NC	22
Suberea creba	B, *Pseudomonas* sp.	Quinolones	22
Tedania ignis	B, *Micrococcus* sp.	Diketopiperazines	107
Theonella swinhoei	B, δ-*Proteobacteria*	NC	97
Theonella swinhoei	C, *Aphanocapsa feldmanni*	NC	7
Theonella swinhoei	Filamentous bacteria	Theopalauamide	97
Theonella swinhoei	Unicellular bacteria	Swinholide A	6
Unidentified sponge	A, *Streptomyces* sp.	Urauchimycins A and B	51
Verongia sp.	B, *Aeromonas* sp.	NC	117
Verongia sp.	B, *Pseudomonas* sp.	NC	117
Xestospongia sp.	B, *Micrococcus luteus*	Antimicrobial compounds	9

[a]A, actinomycete; B, Bacteria; C, Cyanobacteria; D, Dinoflagellate; R, Red algae.
[b]NC: It was not checked.

and/or as intracellular symbionts in sponge *Verongia* (117, 124).

The amount of symbiotic microorganisms residing in sponges varies between host species. While bacteria constitute up to 60% of the biomass of some sponges (123, 124, 125), others contain only a small number of bacteria inside their tissue (118). In the case of *Aplysina* sponges, they harbor large amounts of bacteria that constitute about 40% of the biomass and exceed the bacterial concentrations of the seawater by two orders of magnitude (32, 46).

Why do microorganisms live in sponges?

Then why do symbiotic microorganisms inhabit sponges? We can expect putative benefits to symbiotic microorganisms or their hosts. The surfaces or internal spaces of marine sponges are more nutrient-rich than seawater and sediments; therefore sponges offer nourishment and a safe habitat to their symbionts (10). On the other hand, symbiotic microorganisms help in the nutritional process, either by intracellular digestion or by translocation of metabolites including nitrogen fixation, nitrification and photosynthesis (127, 128). Microorganisms also stabilize the sponge skeleton (129) and participate in the host's chemical defense system against predators and biofouling (2, 85, 90, 114). Bacteria collected from sponges have allowed isolation of antimicrobioal compounds, which suggests that these bacteria may play a role in the defence mechanism of these invertebrates (10).

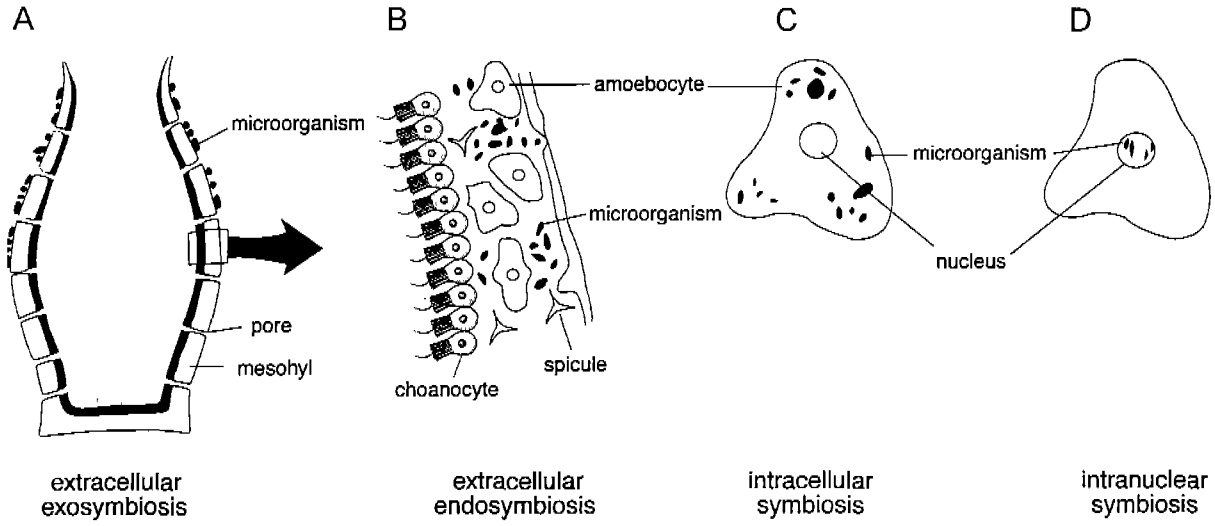

Fig. 1. Schematic diagram of symbiotic relationships between sponges and microorganisms. A, extracellular exosymbiosis; B, extracellular endosymbiosis; C, intracellular symbiosis; and D, intranuclear symbiosis.

How do microorganisms live in sponges?

How can a specific microorganism inhabit a host sponge? The process of sponge-microorganism symbiosis has not been revealed yet. Webster and Hill (121) suggested several possibilities: host selectively absorbs the specific symbiont; the specific symbiont grows more rapidly than any other symbiotic microorganism; or the host acquires the specific symbiont via vertical transmission from parent sponge to larvae. Sponges seem to acquire the symbiotic microorganisms via the feeding route (22). According to TEM observations, symbiotic microorganisms rarely seem to be digested by the sponge (125). Those microorganisms that resist the digestive process can live in sponge tissues. It is yet unclear how sponges distinguish between symbionts and food. Some experimental data suggest the involvement of lectins in this recognition system (80). A sponge can acquire the symbionts from its parent. Some cyanobacteria are transmitted vertically via the eggs of host sponges (22).

What is the symbiotic process? The molecular basis of the symbiotic relationship between sponge and microorganism is not sufficiently understood. We can analogize it from other symbiotic relationships between marine organisms and symbiotic microorganisms. In the case of squid-luminescent bacterium symbiosis, the bacterium plays an active role in the inoculation process (76). Once the appropriate bacteria arrive at the specific point of colonization, the host must present conditions that permit the symbiotic microorganism to recognize this site and be retained there. The selective process is likely to be mediated by specific receptor-ligand interactions; there is evidence that surface glycans function to recognize the specific symbiotic microorganism and facilitate their maintenance (76). Once the appropriate bacteria have established themselves in their target tissues, both the host and the symbiotic microorganism change from an initiation mode to a persistence mode. The bacteria induce significant remodeling of the tissues including apoptosis induced by the lipopolysaccharide (31). The developmental events are triggered by the symbiont within hours of onset of the symbiosis and conspicuous morphological changes ensue over the first few days (76). Further studies on morphological changes during symbiosis and molecular mechanisms of cell recognition will contribute to understanding the symbiotic process of sponges and microorganisms.

Natural products from sponge-symbiotic microorganisms

Marine microorganisms are good candidates for new pharmaceuticals and bioactive natural products (63). There is accumulating evidence that demonstrates the involvement of symbiotic microorganisms in the natural products originally attributed to the sponge host (64, 103, 114). For example, symbiotic bacterium *Micrococcus* sp. produces diketopiperazines previously ascribed to the host sponge *Tedania ignis* (107). Another symbiotic bacterium *Vibrio* sp. produces brominated biphenyl ethers formerly attributed to the host sponge *Dysidea* sp. (24). A dinoflagellate *Prorocentrum lima* produces okadaic acid, first isolated from the host sponge *Halichondria okadai* (63). Symbiotic bacterium *Vibrio* sp. produces an anti-*Bacillus* peptide andrimid that was found in the sponge *Hyatella* sp. extract (82).

Reports on new natural products produced by symbiotic microorganisms have recently increased. Unusual polychlorinated compounds are located in a symbiotic cyanobacterium and antibiotic polybrominated biphenyl ethers are produced by the endosymbiotic cyanobacterium *Oscillatoria spongeliae*, which is hosted by the host sponge *Dysidea herbacea* (30, 115). Cytotoxic macrolide swinholide A is found in the mixed population of unicellular het-

erotrophic bacteria, and an antifungal cyclic peptide theonegramide in the filamentous heterotrophic bacteria (6, 7). Neuroactive compounds are detected in *Antarcticum vesiculatum* and *Psychroserpens burtonensis* isolated from the sponge *Halichondria panicea* (86). Quinolones are found in a pseudomonad isolated from the sponge *Suberea creba* (21). Antimicrobial activities are detected in *Micrococcus luteus* isolated from the sponge *Xestospongia* sp. (9). Antimicrobial compounds such as quinolones and a phosphatidyl glyceride are isolated from a *Pseudomonas* sp. collected at the surface of the sponge *Homophymia* sp. (10).

More bioactive natural products will be discovered from sponge-symbiotic microorganisms. Particularly, the involvement of microorganisms is suspected in these cases: when one sponge species contains different classes of metabolites; when taxonomically different sponges contain the same metabolite; when free-living microorganisms produce identical or similar metabolites; or when the metabolite concentrations are exceedingly low (46, 114). Taxonomic and biochemical information on these symbiotic microorganisms will help in finding new natural products. Furthermore, knowing the biosynthetic pathway of a natural product can help to develop a chemical or biotechnological synthetic method (84).

Laboratory culture and identification of symbiotic microorganisms

The culture of symbiotic microorganisms has important implications for the screening and production of symbiont-derived bioactive natural products (121). Isolation of interesting symbiotic microorganisms is the first step of a laboratory culture. Symbiotic microorganisms can be separated from sponge cells by differential centrifugation (36), by density gradient centrifugation (37), by Ficoll/Percoll density gradient centrifugation (30), or by Ficoll gradient centrifugation (116, 121). Flowers *et al.* (30) showed that the Percoll procedure gives better separation than Ficoll procedure.

Some heterotrophic bacteria are isolated and cultured for metabolite extraction. A *Micrococcus* species from the sponge *Tedania ignis*, an unidentified bacterium from the sponge *Dysidea* sp., and a pseudomonad from the sponge *Suberea creba* were isolated and cultured (21, 24, 107). Autotrophic symbionts such as photosynthetic algae also have been isolated from sponge tissue. Price *et al.* (89) cultivated the red macroalga *Ceratodictyon spongiosum* isolated from the sponge *Sigmadocia symbiotica*, and Hinde *et al.* (47) cultivate the cyanobacterium *Oscillatoria spongeliae* isolated from the sponge *Dysidea herbacea*.

For more effective cultivation, several culture media have been developed. *Vibrio* sp. from the sponge *Hyatella* sp. was cultured in marine agar (82), and various symbiotic bacteria from the sponge *Halichondria panicea* were cultured separately in marine medium 2216 (86). The filamentous symbiotic bacteria from the sponge *Theonella swinhoei* were cultured on agar plates containing aqueous sponge extract (97). After over 100 attempts to culture the symbionts, Schmidt *et al.* (97) could cultivate some filamentous bacteria using J agar with sodium thiosulfate, aqueous sponge extract, and sodium silicate. To improve recoverability of microorganisms from natural marine sponges, Olson *et al.* (83) added catalase or sodium pyruvate to solid growth and isolation media. By adding sponge extracts to marine agar 2216, Webster *et al.* (122) isolated bacterial isolates not previously cultured.

In spite of these successful cultures, most symbiotic microorganisms are difficult to isolate and cultivate (98). Recent data show that only less than 0.1% of the total bacterial community was amenable to culture (121). Because most symbiotic microorganisms cannot be cultured using current and traditional techniques, culture-based techniques are inadequate for studying microbial diversity and identifying unculturable symbiotic microorganisms. To overcome this problem, symbiotic microorganisms are identified directly using culture-independent methods such as molecular taxonomy. Phylogenetic analysis of 16S rRNA genes gives data on microbial diversity and the phylogenetic position of each symbiotic microorganism (121, 122). Innovative culture techniques should be explored to obtain additional sponge-symbiotic microorganisms in cultures. These potentially novel and diverse isolates would be a useful resource for screening for bioactive natural products.

Perspectives

There are several potential drawbacks for future studies on sponge-symbiotic microorganisms and related natural products. First, identification and isolation of symbiotic microorganisms producing bioactive natural products are a crucial step for the future culture and production of these metabolites (116). Second, the origin of natural products can be assigned to symbiotic microorganisms only when synthesis has been demonstrated in cultures isolated from the host species (28). Third, the cultures for metabolites production are uncertain about whether or not the symbiotic microorganism will continue to produce the target compound in the absence of the sponge host (114). These drawbacks can be overcome by the molecular approach. Molecular cloning and analysis of the genes responsible for synthesizing the target compound also offer useful information for genetic engineering of the biosynthesis. In addition, biochemical research on bioactive natural products can contribute to understanding the biosynthesis of the natural products. *In situ* hybridization with specific probes allows the direct, cultivation-independent analysis of most natural symbiotic microbial communities. FISH (fluorescence *in situ* hybridization) based on the 16S rRNA can visualize the localization and

abundance of a specific symbiont and the composition of the symbiotic microorganisms (32, 121, 122). These studies could yield new insights in the true abundance of well-known or new symbiotic microorganisms and increase our knowledge on the species diversity and specificity of symbiotic microorganisms. But the sequence variation among 16S rRNA genes is so small that it is inadequate to distinguish exact species at the level of species or strain. To resolve this low level of sequence variation, more variable sequences such as the ITS (internal transcribed spacer) region can be used, because it contains genetic variation sufficient for differentiating species of microorganisms (3). To identify diversity among symbiotic microorganisms, RFLP (restriction fragment length polymorphism) using arbitrary primers or PCR fingerprinting using repetitive sequences can be applied (20).

Detection of the origin of natural product synthesizing organisms is also an important step. Some specific natural products can be detected directly using fluorescence, therefore the localization of the metabolites can be observed *in situ* (95, 114). Salomon *et al.* (95) detected dercitamide in cells using a combination of visualization methods, including laser-scanning confocal, epifluorescence, and transmission electron microscopy, as well as cell-separation techniques, and chemical analysis. Development of *in situ* detection methods can help identification of the cell type producing bioactive natural products.

Acknowledgments

This work was supported by Life Phenomena & Function Research Project 01-J-LF-01-B-31 of the Korean Ministry of Science and Technology.

References

1. Althoff, K., C. Schütt, R. Steffen, R. Batel, and W.E.G. Müller. 1998. Evidence for a symbiosis between bacteria of the genus *Rhodobacter* and the marine sponge *Halichondria panicea*: harbor also for putatively toxic bacteria? *Mar. Biol.* 130, 529-536.
2. Bakus, G.J., N.M. Targett, and B. Schulte. 1986. Chemical ecology of marine organisms: an overview. *J. Chem. Ecol.* 12, 951-987.
3. Barry, T., G. Colleran, M. Glennon, L.K. Dunican, and F. Gannon. 1991. The 16S/23S ribosomal spacer region as a target for DNA probes to identify eubacteria. *PCR Methods Appl.* 1, 51-56.
4. Bergmann, W. and R.J. Feeney. 1950. The isolation of a new thymine pentoside from sponges. *J. Am. Chem. Soc.* 72, 2809-2810.
5. Bernan, V.S., D.M. Roll, C.M. Ireland, M. Greenstein, W.M. Maiese, and D.A. Steinberg. 1993. A study on the mechanism of action of sceptrin, an antimicrobial agent isolated from the South Pacific sponge *Agelas mauritiana*. *J. Antimicrob. Chemother.* 32, 539-550.
6. Bewley, C.A., H.Y. He, D.H. Williams, and D.J. Faulkner. 1996a. Aciculitins A-C: cytotoxic and antifungal cyclic peptides from the lithistid sponge *Aciculites orientalis*. *E. J. Am. Chem. Soc.* 118, 4314-4321.
7. Bewley, C.A., N.D. Holland, and D.J. Faulkner. 1996b. Two classes of metabolites from *Theonella swinhoei* are localized in distinct populations of bacterial symbionts. *Experientia* 52, 716-722.
8. Bubb, M.R., A.M. Senderowicz, E.A. Sausville, K.L. Duncan, and E.D. Korn. 1994. Jasplakinolide, a cytotoxic natural product, induces actin polymerization and competitively inhibits the binding of phalloidin to F-actin. *J. Biol. Chem.* 269, 14869-14871.
9. Bultel-Poncé, V., C. Debitus, J.P. Berge, C. Cerceau, and M. Guyot. 1998. Metabolites from the sponge-associated bacterium *Micrococcus luteus*. *J. Mar. Biotechnol.* 6, 233-236.
10. Bultel-Poncé, V., J.P. Berge, C. Debitus, J.L. Nicolas, and M. Guyot. 1999. Metabolites from the Sponge-Associated Bacterium *Pseudomonas* Species. *Mar. Biotechnol.* 1, 384-390.
11. Cafieri, F., E. Fattorusso, and O. Taglialatela-Scafati. 1998. Novel betaines from the marine sponge *Agelas dispar*. *J. Nat. Prod.* 61, 1171-1173.
12. Carbonelli, S., A. Zampella, A. Randazzo, C. Debitus, and L. Gomez-Paloma. 1999. Sphinxolides e-g and reidispongiolide c: four new cytotoxic macrolides from the new caledonian lithistida sponges *N. superstes* and *R. coerulea*. *Tetrahedron* 55, 14665-14674.
13. Carmely, S., M. Roll, Y. Loya, and Y. Kashman. 1989. The structure of eryloside A, a new antitumor and antifungal 4-methylated steroidal glycoside from the sponge *Erylus lendenfeldi*. *J. Nat. Prod.* 52, 167-170.
14. Carney, J.R., P.J. Scheuer, and M. Kelly-Borges. 1993. A new bastadin from the sponge *Psammaplysilla purpurea*. *J. Nat. Prod.* 56, 153-157.
15. Casapullo, A., G. Bifulco, I. Bruno, and R. Riccio. 2000. New bisindole alkaloids of the topsentin and hamacanthin classes from the Mediterranean marine sponge *Rhaphisia lacazei*. *J. Nat. Prod.* 63, 447-451.
16. Chang, C.W.J., A. Patra, D.M. Roll, P.J. Scheuer, G.K. Matsumoto, and J. Clardy. 1984. Kalihinol-A, a highly functionalized diidocyano diterpenoid antibiotic from a sponge. *J. Am. Chem. Soc.* 106, 4644-4646.
17. Cimino, G., S. De Stefano, and L. Minale. 1974. Occurrence of hydroxyhydroquinone and 2-aminoimidazole in sponges. *Comp. Biochem. Physiol. B.* 47, 895-897.
18. Copp, B.R., C.M. Ireland, and L.R. Barrows. 1992. Psammaplysin C: a new cytotoxic dibromotyrosine-derived metabolite from the marine sponge *Druinella* (= *Psammaplysilla*) *purpurea*. *J. Nat. Prod.* 55, 822-823.
19. Crew, P., L.V. Manes, and M. Boehler. 1986. Jasplakinolide, a cyclodepsipeptide from the marine sponge, *Jaspis* sp. *Tetrahedron Lett.* 27, 2797-2800.
20. de Bruijn, F.J. 1992. Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergeneric consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. *Appl. Envir. Microbiol.* 58, 2180-2187.
21. Debitus, C., G. Guella, I.I. Mancini, J. Waikedre, J.P. Guemas, J.L. Nicolas, and F. Pietra. 1998. Quinolones from a bacterium and tyrosine metabolites from its host sponge, *Suberea creba* from the Coral Sea. *J. Mar. Biotechnol.* 6, 136-141.

22. Duglas, A.E. 1994. Symbiotic interactions. Oxford University Press, Oxford. pp. 148.
23. Eder, C., P. Proksch, V. Wray, R.W. van Soest, E. Ferdinandus, L.A. Pattisina, and S. Sudarsono. 1999. New bromopyrrole alkaloids from the Indopacific sponge *Agelas nakamurai*. *J. Nat. Prod.* 62, 1295-1297.
24. Elyakov, G.B., T. Kuznetsova, V.V. Mikhailov, I.I. Maltsev, V.G. Bionov, and S.A. Fedoreyev. 1991. Brominated diphenyl ethers from a marine bacterium associated with the sponge *Dysidea* sp. *Experintia* 47, 632-633.
25. Fathi-Afshar, R., T.M. Allen, C.A. Krueger, D.A. Cook, A.S. Clanachan, R. Vriend, H.P. Baer, and C.E. Cass. 1989. Some pharmacological activities of novel adenine-related compounds isolated from a marine sponge *Agelas mauritiana*. *Can. J. Physiol. Pharmacol.* 67, 276-281.
26. Fattorusso, E., L. Minale, and G. Sodano. 1972. Aeroplysinin-1, an antibacterial bromo-compound from the sponge *Verongia aerophoba*. *J. Chem. Soc.* 1, 16-18.
27. Faulkner, D.J. 2001. Marine natural products. *Nat. Prod. Rep.* 18, 1-49.
28. Faulkner, D.J., M.K. Harper, C.E. Salomon, and E.W. Schmidt. 1999. Localisation of bioactive metabolites in marine sponges. *Mem. Queensl. Mus.* 44, 167-173.
29. Fernandez, R., M. Dherbomez, Y. Letourneux, M. Nabil, J.F. Verbist, and J.F. Biard. 1999. Antifungal metabolites from the marine sponge *Pachastrissa* sp.: new bengamide and bengazole derivatives. *J. Nat. Prod.* 62, 678-680.
30. Flowers, A.E., M.J. Garson, R.I. Webb, E.J. Dumdei, and R.D. Charan. 1998. Cellular origin of chlorinated diketopiperazines in the dictyoceratid sponge *Dysidea herbacea* (Keller). *Cell Tissue Res.* 292, 597-607.
31. Foster, J.S., M.A. Apicella, M.J. McFall-Ngai. 2000. *Vibrio fischeri* lipopolysaccharide induces developmental apoptosis, but not complete morphogenesis, of the *Euprymna scolopes* symbiotic light organ. *Dev. Biol.* 226, 242-254.
32. Friedrich, A.B., H. Merkert, T. Fendert, J. Hacker, P. Proksch, and U. Hentschel. 1999. Microbial diversity in the marine sponge *Aplysina cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence *in situ* hybridization (FISH). *Mar. Biol.* 134, 461-470.
33. Fu, X., F.J. Schmitz, R.S. Tanner, and M. Kelly-Borges. 1998a. Agelasines H and I, 9-methyladenine-containing diterpenoids from an *Agelas* sponge. *J. Nat. Prod.* 61, 548-550.
34. Fu, X., F.J. Schmitz, R.S. Tanner, and M. Kelly-Borges. 1998b. New imidazole alkaloids and zinc complexes from the micronesian sponge *Leucetta cf. chagosensis*. *J. Nat. Prod.* 61, 384-386.
35. Fusetani, N., K. Warabi, Y. Nogata, Y. Nakao, S. Matsunaga, and R.R.M. van Soest. 1999. Koshikamide A1, a new cytotoxic linear peptide isolated from a marine sponge, *Theonella* sp. *Tetrahedron Lett.* 40, 4687-4690.
36. Garson, M.J., A.F. Dexter, L.K. Lambert, and V. Liokas. 1992. Isolation of the bioactive terpene 7-deacetoxy-olepupuane from the temperate marine sponge *Dysidea* sp. *J. Nat. Prod.* 55, 364-367.
37. Garson, M.J., M.P. Zimmermann, C.N. Battershill, J.L. Holden, and P.T. Murphy. 1994. The distribution of brominated long-chain fatty acids in sponge and symbiont cell types from the tropical marine sponge *Amphimedon terpenensis*. *Lipids* 29, 509-516.
38. Giannini, C., A. Zampella, C. Debitus, J.-L. Menou, C. Roussakis, and M.V. D'Auria. 1999. Isolation and structural elucidation of the crellastatins i-m: cytotoxic bis-steroid derivatives from the Vanuatu marine sponge *Crella* sp. *Tetrahedron* 55, 13749-13756.
39. Gulavita, N.K., A.E. Wright, P.J. McCarthy, S.A. Pomponi, M. Kelly-Borges, M. Chin, and M.A. Sills. 1993. Isolation and structure elucidation of 34-sulfatobastadin 13, an inhibitor of the endothelin A receptor, from a marine sponge of the genus *Ianthella*. *J. Nat. Prod.* 56, 1613-1617.
40. Gunasekera, S.P., P.J. McCarthy, and M. Kelly-Borges. 1994a. Hamacanthins A and B, new antifungal bis indole alkaloids from the deep-water marine sponge, *Hamacantha* sp. *J. Nat. Prod.* 57, 1437-1441.
41. Gunasekera, S.P., P.J. McCarthy, R.E. Longley, S.A. Pomponi, and A.E. Wright. 1999a. Secobatzellines A and B, two new enzyme inhibitors from a deep-water Caribbean sponge of the genus *Batzella*. *J. Nat. Prod.* 62, 1208-1211.
42. Gunasekera. S.P., P.J. McCarthy, R.E. Longley, S.A. Pomponi, A.E. Wright, E. Lobkovsky, and J. Clardy. 1999b. Discorhabdin P, a new enzyme inhibitor from a deep-water Caribbean sponge of the genus *Batzella*. *J. Nat. Prod.* 62, 173-175.
43. Gunasekera, S.P., S.A. Pomponi, and P.J. McCarthy. 1994b. Discobahamins A and B, new peptides from the Bahamian deep water marine sponge *Discodermia* sp. *J. Nat. Prod.* 57, 79-83.
44. Haygood, M.G., E.W. Schmidt, S.K. Davidson, and D.J. Faulkner. 1999. Symbiosis of marine invertebrate and microorganism. *J. Mol. Microbiol. Biotechnol.* 1, 33-43.
45. He, H., P. Kulanthaivel, and B. J. Baker. 1994. New cytotoxic sesterterpenes from the marine sponge *Spongia* sp. *Tetrahedron Lett.* 35, 7189-7192.
46. Hentschel, U., M. Schmid, M. Wagner, L. Fieseler, C. Gernert, and J. Hacker. 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.
47. Hinde, R., F. Pironet, and M. A. Borowitzka. 1994. Isolation of *Oscillatoria spongeliae*, the filamentous cyanobacterial symbiont of the marine sponge *Dysidea herbacea*. *Mar. Biol.* 119, 99-104.
48. Hirata, Y. and D. Uemura. 1986. Halichondrins-antitumor polyether macrolides from a marine sponge. *Pure Appl. Chem.* 58, 701-710.
49. Hollenbeak, K.H. and F.J. Schmitz. 1977. Aplysinopsin: antineoplastic tryptophan derivative from the marine sponge *Verongia spengelii*. *Lloydia*. 40, 479-481.
50. Hong, T.W., D.R. Jimenez, and T.F. Molinski. 1998. Agelastatins C and D, new pentacyclic bromopyrroles from the sponge *Cymbastela* sp., and potent arthropod toxicity of (-)-agelastatin A. *J. Nat. Prod.* 61, 158-161.
51. Imamura, N., M. Nishijima, K. Adachi, and H. Sano. 1993. Novel antimycin antibiotics, urauchimycins A and B, produced by marine actinomycete. *J. Antibiot.* 46, 241-246.
52. Ishibashi, M., M. Tsuda, Y. Ohizumi, T. Sasaki, and J. Kobayashi. 1991. Purealidin A, a new cytotoxic bromotyrosine-derived alkaloid from the Okinawan marine sponge *Psammaplysilla purea*. *Experientia*. 47, 299-300.
53. Ishida, K., M. Ishibashi, H. Shigemori, T. Sasaki, and J. Kobayashi. 1992. Agelasine G, a new antileukemic alkaloid from

the Okinawan marine sponge *Agelas* sp. *Chem. Pharm. Bull.* 40, 766-767.
54. Jayatilake, G.S., M.P. Thornton, A.C. Leonard, J.E. Grimwade, and B.J. Baker. 1996. Metabolites from an Antarctic sponge-associated bacterium, *Pseudomonas aeruginosa*. *J. Nat. Prod.* 59, 293-296.
55. Jimenez, C. and P. Crews. 1990. Novel marine sponge amino acids, 10. Xestoaminols from *Xestospongia* sp. *J. Nat. Prod.* 53, 978-982.
56. Juagdan, E. G., R. S. Kalidindi, P.J. Scheuer, and M. Kelly-Borges. 1995. Elenic acid, an inhibitor of topoisomerase ii, from a sponge, *Plakinastrella* sp. *Tetrahedron Lett.* 36, 2905-2908.
57. Kashman, Y., A. Groweiss, and U. Shmeuli. 1980. Latrunculin, a new 2-thiazolidinone macrolide from the marine sponge *Latrunculia magnifica*. *Tetrahedron Lett.* 21, 3629-3632.
58. Kashman, Y., G. Koren-Goldshlager, M.D.G. Gravalos, and M. Schleyer. 1999. Halitulin, a new cytotoxic alkaloid from the marine sponge *Haliclona tulearensis*. *Tetrahedron Lett.* 40, 997-1000.
59. Kato, Y., N. Fusetani, S. Matsunaga, and K. Hashimoto. 1988. Calyculins, potent antitumour metabolites from the marine sponge *Discodermia calyx*: biological activities. *Drugs Exp. Clin. Res.* 14, 723-728.
60. Kim, J.S., Y.J. Lim, K.S. Im, J.H. Jung, C.J. Shim, C.O. Lee, J. Hong, and H. Lee.1999a. Cytotoxic polyacetylenes from the marine sponge *Petrosia* sp. *J. Nat. Prod.* 62, 554-559.
61. Kim, D., I.S. Lee, J.H. Jung, and S.I. Yang. 1999b. Psammaplin A, a natural bromotyrosine derivative from a sponge, possesses the antibacterial activity against methicillin-resistant *Staphylococcus aureus* and the DNA gyrase-inhibitory activity. *Arch. Pharm. Res.* 22, 25-29.
62. Kitamura, A., J. Tanaka, I.I. Ohtani and T. Higa. 1999. Echinoclathrines A-C: a new class of pyridine alkaloids from an Okinawan sponge, *Echinoclathria* sp. *Tetrahedron* 55, 2487-2492.
63. Kobayashi, J. and M. Ishibashi. 1993. Bioactive metabolites of symbiotic marine microorganisms. *Chem. Rev.* 93, 1753-1769.
64. Kobayashi, J., O. Murata, and H. Shigemori. 1993. Jaspisamides A-C, new cytotoxic macrolides from the Okinawan sponge *Jaspis* sp. *J. Nat. Prod.* 56, 787-791.
65. Kobayashi, M., J. Tanaka, T. Katori, and I. Kitagawa. 1990. Marine natural products. XXIII. Three new cytotoxic dimeric macrolides, swinholides B and C and isoswinholide A, congeners of swinholide A, from the Okinawan marine sponge *Theonella swinhoei*. *Chem. Pharm. Bull.* 38, 2960-2966.
66. Kobayashi, M., T. Okamoto, K. Hayashi, N. Yokoyama, T. Sasaki, and I. Kitagawa. 1994. Marine natural products. XXXII. Absolute configurations of C-4 of the manoalide family, biologically active sesterterpenes from the marine sponge *Hyrtios erecta*. *Chem. Pharm. Bull.* 42, 265-270.
67. Kohmoto, S., O.J. McConnell, A. Wright, F. Koehn, W. Thompson, M. Lui, and K.M. Snader. 1987. Puupehenone, a cytotoxic metabolite from a deep water marine sponge, *Stronglyophora hartmani*. *J. Nat. Prod.* 50, 336.
68. König, G.M. and A.D. Wright. 1996. Marine natural products research: current directions and future potential. *Planta Med.* 62, 193-211.
69. Koulman, A., P. Proksch, R. Ebel, A.C. Beekman, W. van Uden, A.W. Konings, J.A. Pedersen, N. Pras, and H.J. Woerdenbag. 1996. Cytotoxicity and mode of action of aeroplysinin-1 and a related dienonefrom the sponge *Aplysina aerophoba*. *J. Nat. Prod.* 59, 591-594.
70. Larkum, A.W.D., G.C. Cox, R.G. Hiller, D.L. Parry, and T.P. Dibbayawan. 1987. Filamentous cyanophytes containing phycourobilin and in symbiosis with sponges and an ascidian of coral reefs. *Mar. Biol.* 95, 1-13.
71. Makarieva, T.N., L.K. Shubina, A.I. Kalinovsky, V.A. Stonik, and G.B. Elyakov. 1983. Steroids in Porifera. II. Steroid derivatives from two sponges of the family Halichondriidae. Sokotrasterol sulfate, a marine steroid with a new pattern of side chain alkylation. *Steroids*. 42, 267-281.
72. Mangalindan, G.C., M.T. Talaue, L.J. Cruz, S.G. Franzblau, L.B. Adams, A.D. Richardson, C.M. Ireland, and G.P. Concepcion. 2000. Agelasine F from a Philippine *Agelas* sp. sponge exhibits in vitro antituberculosis activity. *Planta Med.* 66, 364-365.
73. Matsunaga, S., N. Fusetani, and S. Konosu. 1985. Bioactive marine metabolites, IV. Isolation and the amino acid composition of discodermin A, an antimicrobial peptide, from the marine sponge *Discodermia kiiensis*. *J. Nat. Prod.* 48, 236-241.
74. Mayol, L., V. Piccialli, and D. Sica. 1985. Application of 2D-NMR spectroscopy in the structural determination of gracilin B, a bis-nor-diterpene from the sponge *Spongionella gracilis*. *Tetrahedron Lett.* 26, 1253-1256.
75. McCarthy, P.J., T.P. Pitts, G.P. Gunawardana, M. Kelly-Borges, and S.A. Pomponi. 1992. Antifungal activity of meridine, a natural product from the marine sponge *Corticium* sp. *J. Nat. Prod.* 55, 1664-1668.
76. McFall-Ngai, M.J. and E.G. Ruby. 2000. Developmental biology in marine invertebrate symbioses. *Curr. Opin. Microbiol.* 3, 603-607.
77. Minale, L., R. Riccio, and G. Sodano. 1974. Avarol a novel sesquiterpenoid hydroquinone with a rearranged drimane skeleton from the sponge *Disidea avara*. *Tetrahedron Lett.* 15, 3401-3404.
78. Mong, S., B. Votta, H.M. Sarau, J.J. Foley, D. Schmidt, B.K. Carte, B. Poehland, and J. Westley. 1990. 15-Acetylthioxyfurodysinin lactone, isolated from a marine sponge *Dysidea* sp. is a potent agonist to human leukotriene B4 receptor. *Prostaglandins*. 39, 89-97.
79. Munro, M.H., J.W. Blunt, E.J. Dumdei, S.J. Hickford, R.E. Lill, S. Li, C.N. Battershill, and A.R. Duckworth.. 1999. The discovery and development of marine compounds with pharmaceutical potential. *J. Biotechnol.* 70, 15-25.
80. Müller, W.E.G., R.K. Zahn, B. Kurelec, C. Lucu, I. Muller, and G. Uhlenbruck. 1981. Lectin, a possible basis for symbiosis between bacteria and sponges. *J. Bacteriol.* 145, 548-558.
81. Nakamura, Y., M. Kobayashi, H. Nakamura, H. Wu, J. Kobayashi, and Y. Ohizumi. 1987. Purealin, a novel activator of skeletal muscle actomyosin ATPase and myosin EDTA-ATPase that enhanced the superprecipitation of actomyosin. *Eur. J. Biochem.* 167, 1-6.
82. Oclarit, J.M., H. Okada, S. Ohta, K. Kaminura, Y. Yamaoka, T. Iizuka, S. Miyashiro, and S. Ikegami. 1994. Anti-bacillus substance in the marine sponge, *Hyatella* species, produced by an associated *Vibrio* species bacterium. *Microbios*. 78, 7-

16.
83. Olson, J.B., C.C. Lord, and P.J. McCarthy. 2000. Improved Recoverability of microbial colonies from marine sponge samples. *Microb. Ecol.* 40, 139-147.
84. Osinga, R., J. Tramper, and R.H. Wijffels. 1998. Cultivation of marine sponges for metabolite production: applications for biotechnology? *Trends Biotechnol.* 16, 130-134.
85. Paul, V. J. 1992. Chemical defencs of benthic marine invertebrates: ecological roles of marine natural products. Comstock Publishing Associates, Ithaca, New York.
86. Perovic, S., A. Wichels, C. Schütt, G. Gerdts, S. Pahler, R. Steffen, and W.E.G. Müller. 1998. Neuroactive compounds produced by bacteria from the marine sponge *Halichondria panicea*: activation of the neuronal NMDA receptor. *Environmenta Toxicology and Pharmacology* 6, 125-133.
87. Pettit, G.R., M.S. Butler, C.G. Bass, D.L. Doubek, M.D. Williams, J.M. Schmidt, R.K. Pettit, J.N. Hooper, L.P. Tackett, and M.J. Filiatrault. 1995. Antineoplastic agents, 326. The stereochemistry of bastadins 8, 10, and 12 from the Bismarck archipelago marine sponge *Ianthella basta*. *J. Nat. Prod.* 58, 680-688.
88. Preston, C.M., K.Y. Wu, T.F. Molinski, and E.F. DeLong. 1996. A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. *Proc. Natl. Acad. Sci.* USA. 93, 6241-6246.
89. Price, I.R., R.L. Fricker, and C.R. Wilkinson. 1984. *Ceratodictyon spongiosum* (Rhodophyta), the macroalgal partner in an alga-sponge symbiosis, grown in unialgal culture. *J. Phycol.* 20, 156-158.
90. Proksch, P. 1994. Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs. *Toxicon* 32, 639-655.
91. Quinn, R.J., R.P. Gregson, A.F. Cook, and R.T. Bartlett. 1980. Isolation and synthesis of 1-methylisoguanosine, a potent pharmacologically active constituent from the marine sponge *Tedania digitata*. *Tetrahedron Lett.* 21, 567-568.
92. Rodriguez, J., R.M. Nieto, and P. Crews. 1993. New structures and bioactivity patterns of bengazole alkaloids from a choristid marine sponge. *J. Nat. Prod.* 56, 2034-2040.
93. Ryu, G., B.W. Choi, B.H. Lee, K.-H. Hwang, U.C. Lee, D.S. Jeong, and N.H. Lee. 1999. Wondosterols A-C, three steroidal glycosides from a Korean marine two-sponge association. *Tetrahedron* 55, 13171-13178.
94. Sakai, R., T. Higa, C.W. Jefford, and G. Bernardinelli. 1986. Manzamine A, a novel antitumor alkaloid from a sponge. *J. Am. Chem. Soc.* 108, 6404-6405.
95. Salomon, C.E., T. Deerinck, M.H. Ellisman, and D.J. Faulkner. 2001. The cellular localization of dercitamide in the Palauan sponge *Oceanapia sagittaria*. *Mar. Biol.* 139, 313-319.
96. Santavy, D.L., P. Willenz, and R. R. Colwell. 1990. Phenotypic study of bacteria associated with the caribbean sclerosponge, *Ceratoporella nicholsoni*. *Appl. Environ. Microbiol.* 56, 1750-1762.
97. Schmidt, E.W., A.Y. Obraztsova, S.K. Davidson, D.J. Faulkner, and M.G. Haygood. 2000. Identification of the antifungal peptide-containing symbiont of the marine sponge *Theonella swinhoei* as a novel δ-proteobacterium, "*Candidatus* Entotheonella palauensis". *Mar. Biol.* 136, 969-977.
98. Schmidt, T.M., E.F. DeLong, and N.R. Pace. 1991. Analysis of a picoplakton community by 16S rRNA gene cloning and sequencing. *J. Bacteriol.* 173, 4371-4378.
99. Schmitz, F.J., R.S. Prasad, Y. Gopichand, M.B. Hossain, and D. van der Helm. 1981. Acanthifolicin, a new episulfide-containing polyether carboxilic acid from extracts of the marine sponge *Pandaros acanthifolium*. *J. Am. Chem. Soc.* 103, 2467-2469.
100. Schmitz, F.J., S.P. Gunasekera, G. Yalamanchili, M.B. Hossain, and D. van der Helm. 1984. Tedanolid: a potent cytotoxic macrolide from the Caribbean sponge *Tedania ignis*. *J. Am. Chem. Soc.* 106, 7251-7252.
101. Scott, V.R., R. Boehme, and T.R. Matthews. 1988. New class of antifungal agents: jasplakinolide, a cyclodepsipeptide from the marine sponge, *Jaspis* species. *Antimicrob. Agents Chemother.* 32, 1154-1157.
102. Seo, Y., K.W. Cho, J.-R. Rho, and J. Shin. 1998. Petrocortynes and petrosiacetylenes, Novel polyacetylenes from a sponge of the genus *Petrosia*. *Tetrahedron* 54, 447-462.
103. Shigemori, H. M.A. Bae, K. Yazawa, T. Sasaki, and J. Kobayashi. 1992. Alteramide A a new tetracyclic alkaloid from a bacterium *Alteromonas* sp. associated with the marine sponge *Halichondria okadai*. *J. Org. Chem.* 57, 4317-4320.
104. Shin, J. Y. Seo, K.W. Cho, and J.-R. Rho. 1998a. Osirisynes A-F, highly oxygenated polyacetylenes from the sponge *Haliclona osiris*. *Tetrahedron* 54, 8711-8720.
105. Shin, J., Y. Seo, and K.W. Cho. 1998b. Five new polyacetylenes from a sponge of the genus *Petrosia*. *J. Nat. Prod.* 61, 1268-1273.
106. Simpson, J.S., M.J. Garson, J.W. Blunt, M.H. Munro, and J.N. Hooper. 2000. Mycalamides C and D, cytotoxic compounds from the marine sponge *Stylinos* species. *J. Nat. Prod.* 63, 704-706.
107. Stierle, A.C., J.H. Cardellina II, and F.L. Singleton. 1988. A marine *Micrococcus* produces metabolites ascribed to the sponge *Tedania ignis*. *Experientia*. 44, 1021.
108. Sumich, L. 1992. An introduction to the biology of marine life. 5th ed. Wm. C. Brrown Buplishers, Dubuque. pp. 449.
109. Takada, N., H. Sato, K. Suenaga, H. Arimoto, K. Yamada, K. Ueda, and D. Uemura. 1999. Isolation and structures of haterumalides na, nb, nc, nd, and ne, novel macrolides from an okinawan sponge *Ircinia* sp. *Tetrahedron Lett.* 40, 6309-6312.
110. Teeyapant, R., H.J. Woerdenbag, P. Kreis, J. Hacker, V. Wray, L. Witte, and P. Proksch. 1993. Antibiotic and cytotoxic activity of brominated compounds from the marine sponge *Verongia aerophoba*. *Z. Naturforsch* [C]. 48, 939-945.
111. Thale, Z., F.R. Kinder, K.W. Bair, J. Bontempo, A.M. Czuchta, R.W. Versace, P.E. Phillips, M.L. Sanders, S. Wattanasin, and P. Crews. 2001. Bengamides revisited: new structures and antitumor studies. *J. Org. Chem.* 66, 1733-1741.
112. Tsuda, M., K. Hirano, T. Kubota, and J. Kobayashi. 1999. Pyrinodemin A, a cytotoxic pyridine alkaloid with an isoxazolidine moiety from sponge *Amphimedon* sp. *Tetrahedron Lett.* 40, 4819-4820.
113. Tsukamoto, S., S. Matsunaga, N. Fusetani, and A. Toh-e. 1999. Theopederins f-j: five new antifungal and cytotoxic metabolites from the marine sponge, *Theonella swinhoei*. *Tetrahedron* 55, 13697-13702.
114. Unson, M.D., N.D. Holland, and D.J. Faulkner. 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.
115. Unson, M.D. and D.J. Faulkner. 1993. Cyanobacterial symbiont synthesis of chlorinated metabolites from *Dysidea herbacea* (Porifera). *Experientia* 44, 1021-1022.
116. Uriz, M. J., X. Turon, J. Galera, and J. M. Tur. 1996. New light on the cell location of avarol within the sponge *Dysidea avara* (Dendroceratida). *Cell Tissue Res.* 285, 519-527.
117. Vacelet, J. 1975. Étude en microscopie électronique de lassociation entre bactérie et spongiaires du genre *Verongia* (Dictyoceratida). *J. Microsc. Biol. Cell* 23, 271-288.
118. Vacelet, J. and C. Danadey. 1977. Electron microscope study of the association between some sponges and bacteria. *J. Exp. Mar. Biol. Ecol.* 30, 301-314.
119. Vassas, A., G. Bourdy, J.J. Paillard, J. Lavayre, M. Pais, J.C. Quirion, and C. Debitus. 1996. Naturally occurring somatostatin and vasoactive intestinal peptide inhibitors. Isolation of alkaloids from two marine sponges. *Planta Med.* 62, 28-30.
120. Venables, D.A., L.R. Barrows, P. Lassota, and C.M. Ireland. 1997. Veiutamine. a new alkaloid from the Fijian sponge *Zyzzya fuliginosa. Tetrahedron Lett.* 38, 721-722.
121. Webster, N.S. and R.T. Hill. 2001. The culturable microbial community of the Great Barrier Reef sponge *Rhopaloeides odorabile* is dominated by an α-Proteobacterium. *Mar. Biol.* 138, 843-851.
122. Webster, N.S., K.J. Wilson, L.L. Blackall, and R.T. Hill. 2001. Phylogenetic diversity of bacteria associated with the marine sponge *Rhopaloeides odorabile. Appl. Environ. Microbiol.* 67, 434-444.
123. Wilkinson, C.R. 1978a. Microbial associations in sponges. I. Ecology, physiology and microbial populations of coral reef. *Mar. Biol.* 49, 161-167.
124. Wilkinson, C.R. 1978b. Microbial associations in sponges. II. Nucerical analysis of sponge and water bacteral polulations. *Mar. Biol.* 49, 169-176.
125. Wilkinson, C.R. 1978c. Microbial associations in sponges. III. Ultrastructure of the insitu associations in coral reef. *Mar. Biol.* 49, 177-185.
126. Wilkinson, C.R. 1987. significance of microbial symbionts in sponge evolution and ecology. Symbiosis 4, 135-146.
127. Wilkinson, C.R. and P. Fay. 1979. Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. *Nature* 279, 527-529.
128. Wilkinson, C.R. and R. Garrone. 1980. Nutrition in marine sponges. Invlovement of symbiotic bacteria in the uptake of dissolved carbon. *In* Smith D. C. and Tiffon Y (eds.), Nutrition in the lower Metazoa. Pergamon Press, Oxford, pp 157-161.
129. Wilkinson, C.R., M. Nowak, B. Austin, and R.R. Colwell. 1981. Specificity of bacterial symbionts in Medietrerran and Great Barrier Reef sponges. *Microb. Ecol.* 7, 13-21.
130. Zabriskie, T.M. and C.M. Ireland. 1989. The isolation and structure of modified bioactive nucleosides from *Jaspis johnstoni. J. Nat .Prod.* 52, 1353-1356.
131. Zampella, A., C. Giannini, C. Debitus, C. Roussakis, and M.V. D'Auria. 1999. New jaspamide derivatives from the marine sponge *Jaspis splendans* collected in Vanuatu. *J. Nat. Prod.* 62, 332-334.