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Microbial symbiosis in marine sponges: Overview on ecological aspects

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Abstract

Sponge-microbe associations involve a diverse range of heterotrophic bacteria, cyanobacteria, facultative anaerobes, unicellular algae, and Archaea. Among the suggested benefits that these symbionts may provide to the sponge include nutrition through direct incorporation of dissolved organic matter in the seawater, nutrition through translocation of photosynthate from symbiotic cyanobacteria, transportation of metabolites through the sponge mesohyl, contribution to sponge structural rigidity, and assistance in chemical defense. The biology of the bacterium-sponge relationship has obtained considerable interest as a source of natural products. Sponges produce diverse and unusual bioactive compounds with a high potentiality for further development as drugs and research tools. However, sometimes the metabolite source is uncertain and determination of its origin is difficult, due basically to complications in microbial culture of symbionts. This overview resumes the state of the art of investigations about sponges and its symbionts.

Key words: Bacteria; sponges; symbiosis.

Simbiosis microbiana en esponjas marinas: Revisión de aspectos ecológicos

Resumen

Las asociaciones simbióticas encontradas en esponjas involucran una diversa gama de microorganismos como bacterias heterotróficas, cianobacterias, anaerobios facultativos, algas unicelulares y Archaea. Los beneficios que estos simbiontes pueden proveer a la esponja son a) una mejora nutricional por la incorporación de materia orgánica disuelta en el agua marina, así como por el traslado de fotosintatos de cianobacterias simbióticas; b) un mejor transporte de metabolitos a través del mesohilo de la esponja, y c) un mejoramiento de la rigidez estructural y de la defensa química ante agentes amenazadores. La relación biológica entre bacterias y esponjas ha obtenido un considerable interés como fuente generadora de productos naturales. Las esponjas son productoras de compuestos bioactivos con gran potencialidad para ser procesados como drogas y como herramientas de investigación. No obstante, la fuente del metabolito es a veces incierta y la determinación exacta de su origen resulta difícil, básicamente debido a complicaciones generadas en los cultivos microbianos de los simbiontes. Esta revisión recopila las actualidades en la investigación acerca de las esponjas y sus simbiontes.

Palabras clave: Bacterias; esponjas; simbiosis.

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Introduction

Microbial symbiosis from sponge includes bacteria, archaea and unicellular eukaryotes such as dinoflagellates (plankton). Sponges (Phylum Porifera) are the oldest metazoans. More than 10,000 sponge species are known nowadays, 98% of them being marine sponges. Sponges are relatively simple multicellular animals. Unlike many other metazoans, they lack a nervous system and have no muscle cells. Hence, they cannot move and depend on food particles suspended in water for feeding. No specialized reproductive, digestive, respiratory, sensory, or excretory organs are found in these animals. Even though sponges are considered as a loose association of cells that function more or less independently, they do display cellular specialization and organization of cells into simple tissues (1). The sponge cells are morphogenetically characterized by their intercellular specificity. Sponges are provided with at least two recognition systems for xenogeneic cells, one system which prevents invasion of cells from other species, and a second one, which supports a symbiotic relationship with bacteria. Most of the sponges live in a spectacular symbiosis with bacteria and other unicellular organisms, which may be present in their mesohyl, or intracellularly in vacuoles or even within the nucleus (2).

Sponges bear a high diversity of unusual bioactive compounds (secondary metabolites), that can be further develop as drugs and research tools. However, there are many difficulties regarding the origin of these natural compounds when sponges are studied in symbiotic relationships. Similar and identical metabolites may arise from parallel or convergent evolution, or also from digestive degradation of standard compounds. Another additional problem that has been formulated is the gene transfer from bacteria to sponge. A high frequency of plasmid transfer in the marine environment amplifies the possibility of lateral gene transfer (3).

Microbial Symbionts in Sponges

There are many nuances of physiological union among organisms in the sea. These unions are common and important for the survival of reef communities. Sponge body provides a suitable matrix for bacteria and algae. It has been reported that sponges live in a symbiotic relationship with one-celled organisms such as prokaryotes (bacteria, primarily cyanobacteria), eukaryotes (zooxanthellae), or zoochlorellae (green symbiotic algae). These organisms occur both at the sponge's extracellular and intracellular levels (4).

Many tropical sponges contain endosymbiotic cyanobacteria that are unicellular or filamentous. The cyanobacteria are either intercellular (in sponge tissue directly below the outer surface) or within vacuoles inside the host cells. The sponge obtains nutrients from the digestion of bacteria or from the excretion of bacterial compounds such as glycerol and nitrogen. Cyanobacterial symbionts are thought to shade sponge tissue and thus protect it from the damaging effects of intense light (5). Other facultative anaerobic symbionts metabolize a wide range of compounds and may be involved in removing waste products while sponges are not circulating water (6). Bacterial population is particularly high in massive sponges with a large mesohyl. Cyanobacteria often coat the outer layers of shallow exposed sponges (3, 6, 7, 8).

The role of symbionts in the production of marine natural products

Sponges are producers of a great variety of natural products, with potent bioactivities and novel molecular structures. Secondary metabolite production can be assigned to symbiotic microorganisms only when synthesis has been demonstrated in cultures isolated from the host species, whereby, it is still possible that these compounds are being synthesized in a parallel way by the host. In many instances, the limited availability of sponge material is a mayor obstacle for the commercial production of bioactive compounds of potential pharmaceutical importance. Nevertheless, metabolite isolation from symbiotic bacteria would overcome these limitations, using large-scale laboratory cultures and providing consistent yields. Thus, the need to harvest sponges from their natural environment would be eliminated (9). However, the culture of microbial symbionts is not a trivial problem. Without cultivable bacteria or taxonomic data derived from molecular biology, the evolutionary significance of the symbiosis is uncertain.

Antimicrobial compounds have been isolated from sponge-associated bacteria in many occasions, suggesting that microbial symbionts play a role in the defense of the host sponge. *Vibrio* sp. associated with the sponge *Dysidea* sp. were shown to synthesize cytotoxic and antibacterial tetrabromodiphenyl ethers; *Micrococcus* sp. associated with the sponge *Tedania* sp. were found to produce diketopiperazines. An antifungal peptide theopalauamide, isolated from the marine sponge *Theonella swinhoei*, was contained in a novel δ-proteobacterial symbiont (*Candidatus Entotheonella palauensis*) (9, 10).

Two associations studied between the sponge *Dysidea herbacea* and the cyanobacterium *Oscillatoria spongeliae* revealed that chlorinated amino acid-derived metabolites were produced by the cyanobacteria whilst the sponge produced terpenes. In a second specimen, it was found that the major secondary metabolites were polybrominated biphenyl ethers and that these were produced by cyanobacteria. Why *O. spongeliae* produced two different classes of halogenated compounds was not understood (11).

Filamentous bacteria associated to the lithistid sponge *Theonella swinhoei* was found to produce complex bicyclic-peptides and a cytotoxic metabolite called swinholide A was encountered in a fraction containing many unicellular heterotrophic bacteria. The presence of microbial symbionts in *T. swinhoei* suggested that swinholide A and many other bioactive cyclic peptides from the sponge were produced by symbiotic cyanobacteria. However, swinholide A was limited to the mixed population of unicellular heterotrophic bacteria, and an anti-fungal cyclic peptide occurred only in the filamentous heterotrophic bacteria (12). De Rosa et al. (13) reported the production of cyclic peptides from marine bacteria associated with the marine sponge *Ircinia variabilis.* Here, bacterial growth was improved using media supplemented with marine derived proteins. The composition of free and total fatty acids of strains cultivated under different carbon source was investigated and different bacterial diketopiperazines were isolated.

Piel et al. (14) reported the biosynthesis of an antitumor polyketide by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei.* They identified an uncultured *Pseudomonas sp.* symbiont as the most likely producer of the defensive antitumor polyketide pederin in *Paederus fuscipes* beetles, by cloning the putative biosynthesis genes. Sequence features of the isolated genes indicated that it belonged to a prokaryotic genome and is presumed to be responsible for the biosynthesis of almost the entire portion of the polyketide structure that is correlated with antitumor activity. Further, using terrestrial *Paederus spp.* beetles and the marine sponge *Theonella swinhoei* as model animals, the putative genes responsible for the production of pederin-type antitumor polyketides were isolated. A phylogenetic approach was used to isolate the onnamide/theopederin polyketide synthase genes from an uncultured sponge symbiont. Analysis of the biosynthesis genes revealed a possible evolution of pederin-type pathways. These first gene clusters from uncultivated symbiotic producers suggested possible biotechnological strategies to solve the supply problem associated with the development of most marine drug candidates (15).

Symbiosis examples

The use of molecular approaches for describing microbial diversity has greatly enhanced the knowledge of population structure in natural microbial communities. It is widely accepted that culture-based techniques are inadequate for studying bacterial diversity from environmental samples, as many bacteria cannot be cultured using current traditional techniques. Cloning and sequencing of 16S rRNA genes has given data used to describe complete microbial community composition, indicating possible nutritional requirements and physiological niches of many microbes based on information already available for known phylogenetic relatives (9, 16).

Using these techniques, the archaeal microorganism *Cenarchaeum symbiosum* was found living specifically within a sponge similar to *Axinella mexicana* at ~10°C, and thus considered psychrophilic (17). In another example, *in situ* hybridization probes specific to Archaea, the subclasses of Proteobacteria, Flavobacteria-Cytophaga, and sulfate reducing bacteria were used to survey the marine sponges *Chondrosia reniformis* and *Petrosia ficiformis* (3). Both sponges contained mainly γ-subclass of Proteobacteria.

Studies on the marine sponge *Rhopaloeides odorabile* determined an extremely diverse community structure, with representatives of the Actinobacteria, low G+C Gram positive bacteria, the β- and γ-subdivisions of the Proteobacteria, Cytophaga / Flavobacterium, green sulfur bacteria, green non-sulfur bacteria, planctomycetes, and other sequence types with no known close relatives, using 16S rRNA sequencing of cloned DNA fragments and fluorescence *in situ* hybridization (9). Another study made in *Rhopaloeides odorabile* showed that the microbial community was dominated by a single bacterium, an α-subgroup of the class Proteobacteria (18).

Cells of bacteria-like symbiont microorganisms were observed within the mesohyl of several sponges (*Stromatospongia*, *Astrosclera*, *Jaspis*, *Preudoceratina* and *Axinyssa*). These bacterial cells exhibited a membrane-bounded nuclear region encompassing the fibrillar nucleoid. A single bilayer membrane, dividing the cell cytoplasm into two distinct regions, bound the nuclear region. The bacterial symbiotic cells showed analogy to the archaeal sponge symbiont *Cenarchaeum symbiosum* (19).

Studies on the sponge *Ceratoporella nicholsoni* found that ~78% of the bacteria present could be classified into four main groups: two *Vibrio sp.*, an *Aeromonas sp.* and a *Coryneforme* or *Actinomyces sp* (20). In another example, *Rhodobacter sp.* was found dominantly living in symbiosis in the mesohyl compartment of the marine sponge *Halichondria panicea*; yet, the potential function of the symbiosis was not established (4).

The importance of heterotrophy on ultraplankton communities as nitrogen source for the sponge-rhodophyte symbiosis has been reported (21). The availability of enough particulate organic nitrogen to support nitrogen needs of an invertebrate-algal symbiotic association is afforded thanks to sponge's grazing on ultraplankton. The natural diet of the sponge in the *Haliclona-Ceratodictyon* association consists of bacteria and protozoans, which are rich sources of nitrogen.

Müller et al. (22) isolated SB2 bacterium from the sponge surface and grew in minimal medium supplemented with protocatechuate. The protocatechuate gene cluster was cloned and sequenced from the SB2 bacterium. The cluster comprised all genes coding for enzymes involved in the protocatechuate conversion to acetyl coenzyme A. When bacteria were cultivated on protocatechuate medium, the expression was strongly induced. They concluded that metabolites (diphenols) -which might be produced by the sponge- are used by the sponge surface-associated bacterium for energy generation.

Usher et al. (23) investigated the distribution, host associations, and phylogenetic relationships of the unicellular cyanobacterial symbionts of selected marine sponges with direct 16S rDNA sequencing. Their results indicated that the symbionts of the marine sponges *Aplysina aerophoba*, *Ircinia variabilis*, and *Petrosia ficiformis* from the Mediterranean, four *Chondrilla* species from Australia and the Mediterranean, and *Haliclona sp.* from Australia carry a diversity of symbionts including at least four closely related species of *Synechococcus*. They found also a fifth not described symbiont from *Cymbastela marshae* in Australia, related to *Oscillatoria rosea*. Another symbiont, *Candidatus Synechococcus spongiarum*, was found in different sponge genera in the Mediterranean Sea and the Indian, Pacific, and Southern oceans. Also 16S rDNA studies community analysis showed that Actinobacteria, exclusively within the sub-class Acidimicrobidae, are the major bacterial community associated with two sponge species in the genus *Xestospongia* (24). Four groups of Actinobacteria in *Xestospongia spp.* were found, including a novel assemblage bacterial group in *X. testudinaria*, distantly related to any previously sequenced actinobacterial clones.

Ecologically related aspects of sponges in the Caribbean

Sponges are a key component in coral reefs ecosystems, mangroves, among others, having important consequences for Caribbean environments. Porifera are among the most prominent taxa in reef ecosystems, generally exceeding in number of species corals and algae (25). Due to difficulties in identification and quantification, sponges have been avoided in the assessment and monitoring of coral reefs. Their functional roles in the marine ecosystem like calcification, cementation and bioerosion processes on coral reefs are being now recognized (26). A review dealing with the ecological interactions of marine sponges has been published (27). The paper emphasizes the competition among sponge species, and between sponges and other sessile organisms, and explains the symbiotic associations of sponges with a variety of organisms.

The fate of Caribbean sponges is closely related to Caribbean coral reefs. Sponges, in particular clionid sponges, are among the most common and destructive endolithic borers on coral-reef ecosystems. Clionid sponges invade many different types of substrate, including carbonate rock, coral skeleton, mollusk shells, and even man-made calcareous structures (28). Excavation mechanisms, growth rates, and environmental conditions of Clionidae were determined to affect space competition between these sponges and corals on shallow Caribbean reefs (29). Unlike other sponges, clionids may kill coral polyps by undermining and eroding their skeletal base, thus avoiding contact with defensive mucus and nematocysts. Many aggressive encrusting sponges contain high densities of photosynthetic symbionts: e.g. *Cliona caribbaea* and *C. varians* were each associated with a different species of dinophycean zooxanthellae, *Chondrilla* cf. *nucula* and *Terpios hoshinota* contained different cyanobacteria (zoocyanellae). However, non-boring encrusting sponges may also overgrow coral by lateral spreading, such as the symbiotic species of Chondrilla and Terpios (with cyanobacteria). Encrusting clionids and other sponges may considerably transform the community structure and physical stability of shallow reefs that are readily compromised by natural or anthropogenic pressures (29). Another study on Cliona (30) interpreted current trends of reef space occupation. Three Caribbean species were studied and their distribution patterns and size determined. Contrary to the effect on other excavating sponges, chronic exposure to raw sewage did not significantly increase the abundance of the studied sponges. Substratum occupation and availability ratios showed a positive tendency of the sponges toward certain coral skeletons, but a negative or neutral tendency toward calcareous rock. This suggested that establishment may be easier on clean, recently dead coral than on older, heavily incrusted substratum (30).

The excavation rate by boring sponges has been determined (31), by developing a system where chemical and mechanical boring rates ($CaCO_3$ dissolution and chip production, respectively) were measured simultaneously in experimental tanks containing reef's rock inhabited by sponge *Pione cf. vastifica* (Clionaidae). Chemical bioerosion rates were measured as an alkalinity increase vs. time, and the mechanical bioerosion rate was estimated from the total amount of $CaCO_3$ chips produced. One mass of chips per 3 masses of dissolved reef $CaCO_3$ framework was determined during the boring activity (31).

Another interesting point to consider is the chemical defense in sponges. Ecological functions of alkaloids production by sponge are not necessarily a response before predators. Some functional roles may include inhibition of fouling and/or infection and mediation of spatial competition (32). It has been found that sponges of the genus *Aplysina* accumulate brominated isoxazoline alkaloids in concentrations that sometimes exceed 10% of their dry weight (33). A concentration drop of these compounds has been reported after injury of sponge tissue from *Aplysina aerophoba*, observing an increase of aeroplysinin-1 and dienone concentration. However, after studying Caribbean *Aplysina* species, it was conclude that tissue damage induced a bioconversion of isoxazoline alkaloids into aeroplysinin-1 and dienone. This biocatalyzed reaction might be ecologically relevant as the bioconversion products possibly protect the wounded sponge tissue from invasion of bacterial pathogens (33).

The sponge *Chondrilla nucula* is involved in ~ 50% of coral-sponge interactions on Caribbean reefs (34). *C. nucula* represents the preferred prey of the Hawksbill turtle and of other several spongivorous fish. As experimentally presented, when sponge predators (mainly angelfish) were excluded, an increase in sponge overgrowth was observed, and loss of coral cover followed. Predation had a greater influence on *C. nucula* than on the other sponges studied. Predator exclusion experiments performed with naturally occurring coral-sponge interactions demonstrated an important reduction in total coral cover. Hence, indirect effects arising from spongivory might have large community consequences; and species diversity on Caribbean reefs might be partly maintained by spongivores (34). Again, this predator diminishing scenario might be ecologically important, for the bioconversion products can probably protect wounded sponge tissue from bacteria invasion. The influence of predation in sponge was also emphasized by Meylan (35). Hawksbills (*Eretmochelys imbricata*) are endangered marine turtles associated with coral reefs throughout the tropics. Hawksbill feeds practically only on sponges in the Caribbean. The article refers that by affecting space competition, spongivory by hawksbills might affect succession and diversity of reef communities. A similar article reviews the impact of hawksbill in the Caribbean with an interesting scope on sponge as food source and impact on the coral reef (36).

Chemical defense of sponges seems to be highly effective against most fish species in order to protect from predators. Becerro et al. (37) worked on the hypothesis that predation is higher in tropical than in temperate habitats. Thus, tropical species evolved more effective defenses to deter predators. Their study provided the first experimental test of latitudinal differences in the effectiveness of sponge chemical defenses. All the sponges investigated showed deterrent properties against some predators. 35% of the sponge species were, however, deterrent in at least one, but not in all the experiments. Hence, the idea that predators could respond to chemical defenses in a species-specific manner was supported. Tropical and temperate sponges had comparable deterrence, fact that suggested not only similar chemical defenses from tropical and temperate sponges, but also that they were equally effective against predators. Apparently, a recurrent selection for chemical defenses in sponges as a general life-history strategy exists (37).

It has been reported that specimens of the spongivorous Mediterranean opisthobranch *Tylodina perversa,* collected while feeding on sponge specie *Aplysina aerophoba* were shown to sequester the brominated isoxazoline alkaloids of their prey (38). A known sponge alkaloid aerothionin, found only in *A. cavernicola* but not in *A. aerophoba,* was also among the metabolites identified in *T. perversa.* This work postulated that Mollusc derived aerothionin is resultant from a previous feeding encounter with *A. cavernicola,* as *T. perversa* was found to freely feed on both Aplysina sponges in aquarium bioassays. The possible ecological roles of sequestered sponge alkaloids in *T. perversa* are still not known. Nevertheless, alkaloids sequestration might represent the origin of frequent food poisoning cases after ingestion of hawksbill flesh (39). Mass food poisoning through ingestion of turtle has been reported (40, 41). Surveys concerning seafood poisonings indicated that all cases were related to marine toxins (42, 43).

A study carried out with Caribbean sponges genus *Ircinia* (44) contained high concentrations of linear furanosesterterpene tetronic acids (FTAs), producing and emanating low-molecular-weight volatile compounds like, dimethyl sulfide, methyl isocyanide, and methyl isothiocyanate. It has been suggested that FTAs are unlikely to function as antipredatory chemical defenses, and this function may instead be attributed to bioactive volatiles (44). Crude organic extracts and purified fractions isolated from *Ircinia campana*, *I. felix*, and *I. strobilina* were assayed at naturally occurring concentrations in laboratory and field feeding assays to determine their palatability to generalist fish predators. A qualitative technique was employed also, to test the crude volatile fraction from *I. felix* and *I. strobilina* and dimethylsulfide in laboratory feeding assays. The results revealed that crude organic extracts of all three species deterred feeding of fishes in both aquarium and field experiments. The work postulated that FTAs, besides playing a likely ecological role in *Ircinia spp.*, are effective as defenses against potential predatory fishes; and that volatile compounds may serve other defensive functions like antimicrobial or antifouling, however defense against fish predators was not provided (44).

The fact that sponges exude volatile aromatic compound might have an impact in climate regulation (45). Dimethylsulfoniopropionate (DMSP) is an important component of the global sulfur cycle and possibly involved -via its cleavage product dimethylsulfide- in climate regulation. The distribution of DMSP in a diverse group of coral reef invertebrates was studied. Though common in many algae, reports of DMSP in tropical invertebrates are few. It has been suggested that DMSP might be dietarily derived in sponges (45). A related work (46), found 59 volatiles when working with *Ircinia felix* extracts, including dimethylsulfide, methyl isocyanide and methyl isothiocyanate. Exudation experiments in aquarium and in situ conditions revealed that thiobismethane, methyl isocyanide and methyl isothiocyanate were continuously released by the sponge, and its concentration increased upon injury. It was proposed that these substances form a chemical protective barrier, helping sponges to avoid fouling, compete for space, prevent short term infection, or signal generalist predators, regarding the existence of other toxic substances in the internal tissues.

Investigations in Venezuela and surroundings

Venezuela and the surrounding Caribbean area bear a rich marine biodiversity, where sponges play an important role. The sponges *Anthosigmella varians*, *Cinachyrella kuenkenthali*, *Ircinia felix*, *I. strobilina*, *Aplysina fistularis fistularis*, *Niphates erecta*, *Amphimedon viridis*, *Mycale (Carmia) microsigmatosa*, *Ulosa ruetzleri* and *Halichondria melanadocia* have been collected and identified at Isla Larga, Mochima Bay, Venezuela (47). Bitter et al. (48) studied the competition among species of sponges grown on artificial substrates at Morrocoy National Park, Venezuela. Their work stated that slow growing species could be more aggressive and competitive for available space. They determined that sponge's assemblages are organized as a network rather than a hierarchical system (48).

The taxonomy of Caribbean excavating sponge species complex *Cliona caribbaea – C. aprica – C. langae* have also been studied (49). To resolve the species taxonomy, observations and sampling in Colombia, Venezuela, Curacao, Belize, Jamaica and Puerto Rico were carried out. Results based on differences in external morphology, color, spicule morphology and size revealed the existence of three distinct species, *Cliona aprica* Pang, 1973, *Cliona caribbaea* Carter, 1882 (junior synonym *C. langae* Pang, 1973), and *Cliona tenuis sp. nov.* Spicule morphology and size showed geographical variation but remained distinctive for each species within a given locality. Studies related to taxonomy of Caribbean sponges have been published elsewhere (50).

Reports correlated with secondary metabolites are varied and some are briefly cited. The molecule (-)-(5S)-2-imino-1--methylpyrrolidine-5-carboxylic acid has been isolated from organic extracts of the burrowing sponge *Cliona tenuis* (51). Another example is the three sesquiterpenes obtained from an extract of the Caribbean marine sponge *Axinyssa ambrosia*, namely (4R*,5R*,7S*,10R*)-4-isocyanatoeudesm--11-ene; (4R*,5R*,7S*,10R*) -formami doeudesm-11-ene, and (4R*,5R*, 7S*,10R*)-eudesm-11-en-4-ylamine hydrochloride, the latter with significant cytotoxic activity against cancer cells, and active in lethality tests using polyps of the scleractinian coral *Madracis mirabilis* (52).

Agelasines, natural compounds isolated from marine sponges *Agelas sp.*, showed cytotoxic activity over the breast cancer cell line MCF-7 (53). Agelasines are monocyclic diterpenes, bearing a 9-methyladenine group. They have been considered as secondary metabolites with an antimicrobial action, as elsewhere reported (54). Palacios et al. (55) determined the effect of agelasine B, agelasine J, and sceptrine, extracted from *Agelas sp.* over the production of reactive oxygen species (ROS) and nitrogen (RNS) and the viability of muride macrophage RAW 264.7 and human polimorphonuclear leucocytes. They concluded that agelasine B and sceptrin inhibit NO production in muride macrophages, and consequently could be possible anti-inflammatory products (55). Another work reports the isolation from *Agelas sventres* of the new compound sventrin (56), acting as a feeding deterrent compound against a common omnivorous reef fish. Related studies have been reported (57). Barroeta et al. (58) dealt with the effect of ilimaquinone, 11-deoxyfistularin-3 and fistularin-3, isolated from marine sponges, over the production of reactive oxygen species and viability of human polimorphonuclear leucocytes. It was concluded that 10 mM ilimaquinone was the most reactive specie, whilst other compounds tested were not able to inhibit superoxide generation. These results correspond to some conjectures done in previous reports (59, 60).

Some other reports found, deal more with enzymatic activities present in sponge extracts. Recently, a serine protease activity was isolated from *Geodia cydonium* and *Suberites domuncula* and analyzed by two dimensional zymography (61). Conversely, a phospholipase activity present in extracts from *Cinachyrella kuekenthali* has also been reported (62).

Final Remarks and Future Perspectives

Bacteria and other microorganisms play an important role in the sponge's life by processing waste products, transfer of nutrients, and production of secondary metabolites. Many associations between marine sponges and microorganisms are quite specific and considered as symbiotic associations. Endosymbionts contained in marine sponges include bacteria, dinoflagellates, diatoms, and cryptomonads. Microbial symbionts produce natural products that sometimes had been attributed to their hosts, and hence were proposed to contribute to the chemical defense of the association. Determination of the origin of biosynthetic source of natural products must be approached through complementary cell dissociation and chemical analysis. *In situ* techniques have been developed to establish the exact origin of metabolites. Techniques for molecular survey are now standard in microbial ecology, and can be applied to symbiotic systems. rRNA genes are frequently used due to the large database available and the inherent phylogenetic utility of these genes. Sequences of amplified genes reveal the phylogenetic groups, to which the organisms belong and can provide specific probes for *in situ* hybridization, allowing sequences to be linked to specific cells. Thus, a great potential is accomplished in understanding sponge symbiosis. The study of symbiosis represent a key step for enhancing the availability of several natural products of pharmaceutical interest, which otherwise would be inaccessible. The literature over sponges related with ecological and pharmaceutical aspects is vast and contains innumerable examples of the importance of these as potent pharmaceutical targets, with applications in biotechnology, molecular biology, ecology and natural products chemistry. Not all of the literature existing could be cited in this review. New specie discoveries might bring more information over ecological relevant aspects in reef communities. Many of the chemicals secreted by sponges still need to be analyzed and characterized, and its biological, ecological and biochemical significance studied.

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