

Review article.

Sponge-bacteria association: A useful model to explore symbiosis in marine invertebrates

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Abstract

Marine organisms are well known to have specific relationships with numerous microorganisms, and sponges are no exception to this. The studies of these associations underscore the importance of marine sponges as living fossils as well as their significance in drug discovery. The present state of our knowledge on sponge-bacteria associations is based mainly on the culture of associated microorganisms from the sponges. However, molecular biological approaches are also being used to expand our understanding regarding the phylogenetic relationships between the bacteria and their sponge hosts. The increasing number of reports about sponge-bacterial associations highlights the importance of this subject. Several investigations have reported phylogenetically complex yet uniform bacterial associations in marine sponges. It is now clear that at least some of these associations should be regarded as symbioses in view of studies of both the partners with the special emphasis on benefits of this association to both. Finally, comprehensive investigations on sponge-bacteria association show this alliance to be a useful model for exploring symbiosis in marine invertebrates.

Keywords: Marine sponge, bacteria, symbiosis, molecular approach, transmission of symbionts

1. Marine Sponges

Marine sponges (Porifera) are the oldest metazoan group and they have outstanding importance as living fossils (Müller, 1998; Müller, 2003a). There are approximately 8,000 described species of sponges and perhaps twice as many un-described species (Hooper and Van Soest, 2002). They are grouped into three classes, the Hexactinellida (glass sponges), the Calcarea (calcareous sponges) and the Demospongiae. The latter class contains the vast majority of extant sponges living today. Sponges inhabit every type of aquatic environment, from polar seas to temperate and tropical waters and also thrive and prosper at all depths. They show an amazing variety of shapes, sizes and colors (Fig. 1). Giant barrel sponges can reach up to 1.5 meter in height, while the tiny encrusting sponge may only be 2 cm long. Sponges are sessile organisms; however, due to their cellular plasticity, many sponges reorganize their bodies continuously and move during this process very slowly (Bond, 1992). Except for the free swimming larval stage (by

means of cilia), sponges pass their whole subsequent existence fixed to a suitable substratum.

Sponges contain certain unifying attributes that are characteristic for the phylum Porifera. The organization of the sponge body is very simple. Epithelial cells (Pinacocytes) line the outer surface and the internal system of openings, channels and chambers, through which water is pumped continuously by flagellated cells called choanocytes. These simplest animals are very efficient filter-feeders. It has been estimated that some of them are able to filter their own body volume of water every 5 seconds (Vogel, 1977). This water current supplies food particles and oxygen and removes metabolic waste products. The major part of sponge biomass consists of a gelatinous matrix containing free-floating, non-differentiated cells. This part of the sponge body is called the mesohyl. The mesohyl also contains the skeletal elements of the sponge body; spicules (needle like structures made of either silicon or calcium carbonate) and spongin (collagenous fibers).

2. Sponge-Bacteria Association

Marine organisms are well known to have specific

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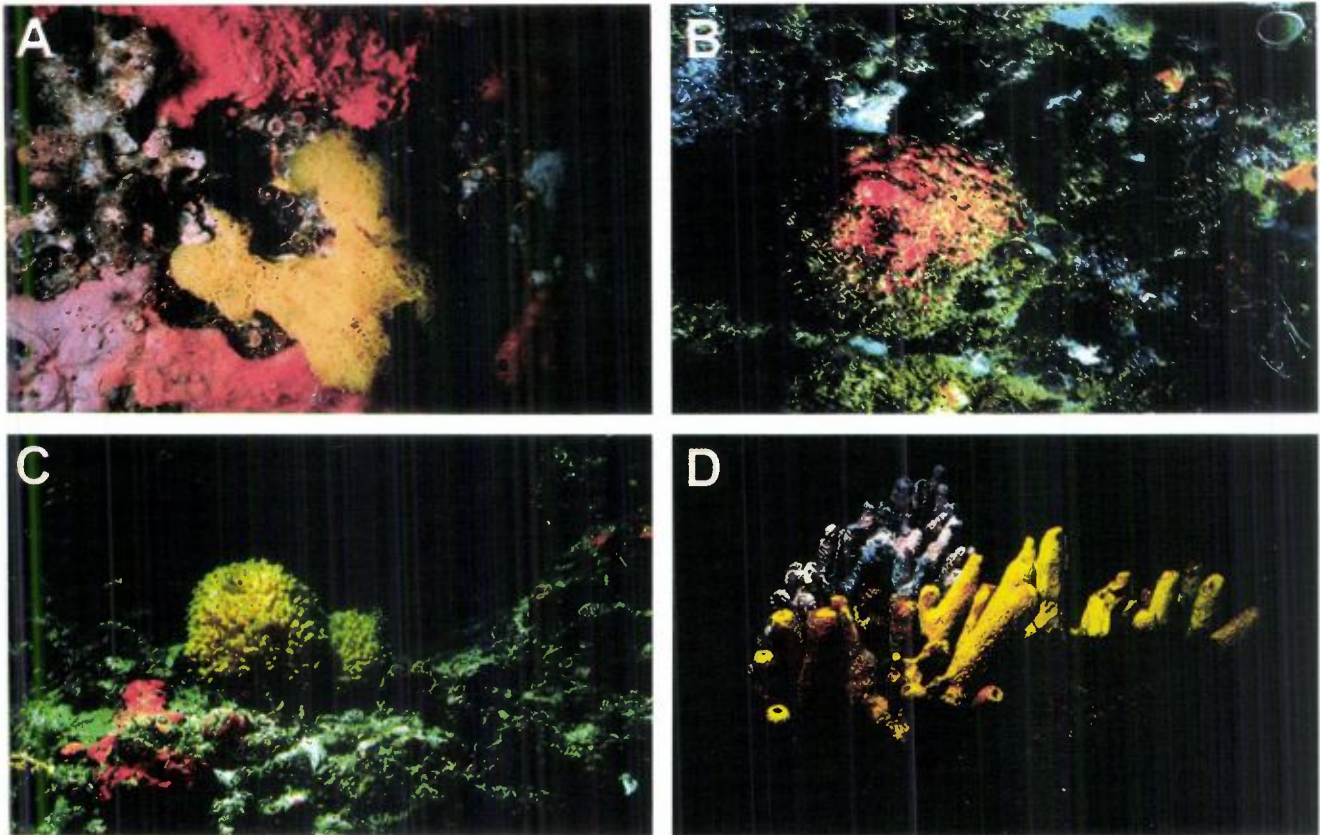


Figure 1. Different sponge species: A, the calcareous sponge *Clathrina coreacea*; B, the siliceous sponge *Tethya limski*; C, *Tethya lyncurium*, a close relative of the red sponge; D, the yellow horny demosponge *Verongia aerophoba* and the pink *Dysidea avara*; both sponges contain bioactive compounds that have been extensively studied.

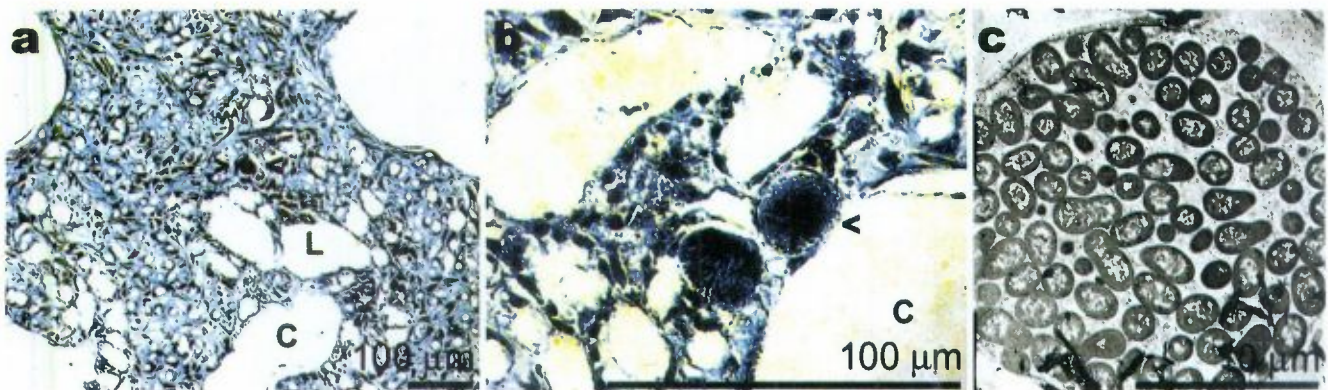


Figure 2. Presence of bacteria in the sponge *Suberites domuncula*. Light micrograph of a semi-thin section through the tissue of this sponge. In a it is shown that the bacteriocyte (marked by two arrow heads) is found in the mesohyl compartment adjacent to lacunae (L), which are surrounded by an epithelium formed by endopinacocytes. In b bacteriocytes (arrow head) are shown which are embedded in the epithelium which surrounds a water canal (C). c shows a transmission electron micrograph through a bacteriocyte which is almost completely filled by bacteria. Scale bars: a: 100 µm; b: 100 µm; c: 10 µm.

relationships with numerous microorganisms and sponges are no exception to this. Association of sponges with microorganisms probably dates back to Precambrian times about 500 million years ago (Wilkinson, 1984). A wide range of organisms are symbiotic with marine sponges, with the most commonly identified being heterotrophic

bacteria (Santavy et al., 1990; Wilkinson, 1978a,b,c) and cyanobacteria (Vacelet, 1971; Usher et al., 2001; Usher et al., 2004). During the feeding process, bacteria from the surrounding seawater are continuously swirled into the sponge, driven by currents, and most of these bacteria are retained in the sponge body and eventually digested. Several

different morpho-types of bacteria are often found in a given host. Interestingly, the bacterial density in sponges varies seasonally in concert with that in the surrounding environment (Thakur, 2001) and bacterial density is also linked to water movement through the sponge (Imhoff and Stöhr, 2003). Sponges with a poor water circulation system appear to have high bacterial numbers whereas well irrigated sponges appear to contain fewer bacteria within their tissues (Vacelet and Donadey, 1977; Wilkinson, 1978a).

It has been estimated that, in some sponge species like *Aplysina cavernicola*, as much as 40% of the animal biomass can be attributed to the bacteria, exceeding the bacterial density of seawater by two to four orders of magnitude (Friedrich et al., 2001). Apart from this, it has been found that some bacteria reside permanently in the sponge mesohyl, pointing to a close interaction between the host and associated bacteria (Fig. 2) (Webster and Hill 2001; Friedrich et al., 2001; Böhm et al., 2001). It is important to note that Fieseler et al. (2004) have recently proposed a new candidate phylum named 'Poribacteria' to acknowledge the specific affiliation of some new bacteria with sponges.

Scientists have been fascinated by the nature of sponge-bacterial associations for a long time. However, due to the complexity of this association, progress in understanding the interaction between these two partners has been difficult and slow (Imhoff and Stöhr, 2003). Even though most of the bacteria in the sponge mesohyl that occur extracellularly or inside the bacteriocyte cells are often addressed as symbionts, the question as to whether all the sponge-associated bacteria should be considered as symbionts has often been debated in the literature (Hentschel et al., 2003). Symbiotic relationships are defined as mutualistic interactions with positive effects for both partners. Although clear hints for such close interactions between microorganisms and sponges exist in some special cases, for the majority of bacteria found in sponges, the specific beneficial interrelationship has not been demonstrated. It is likely that the ecology of sponges and their associated bacteria are independent, yet we know very little about this association in terms of species relationship and distribution.

In view of the difficulties in proving the symbiotic relationship, all of the bacteria isolated from sponges are termed as sponge-associated bacteria.

3. Transmission of Bacterial Symbionts

In a classical symbiosis, the transmission of symbionts between generations is usually classified as either horizontal (environmental) or vertical (transovarian) (Nyholm and McFall-Nagi, 2004). In a horizontally transmitted symbiosis, each new generation of the host acquires the symbiont from the surrounding environment. However, in a vertically transmitted symbiosis the microbial partner is passed on to the next generation via the reproductive stages. Even though both types of associations are widely

distributed across phyla, horizontal "transmission" of symbiosis is thought to be the most common one in animals. Interestingly, there are a few reports on vertical and horizontal transmission of bacterial symbionts in some sponges (Douglas, 1994; Friedrich et al., 1999).

While studying the vertical transmission of bacterial symbionts in the marine sponge *Chondrilla australiensis* (Demospongiae), it was observed that sometimes symbionts are passed on to the next generation of sponges via the eggs (Usher et al., 2001). A similar observation was also been made by Vacelet (1975) in *Ircinia oros*, with some embryos containing bacteria from the female parent and others not. The existence of symbiotic bacteria within sponges is supported by the observation that *Spongia* and *Hippospongia* spp. transfer bacteria from the parent to the embryo (Simpson, 1984; Kaye, 1991). Such transfer and re-establishment of a new bacterial population in daughter sponges highlights the importance of continued association and does indicate a specific and, possibly, a symbiotic association. Unfortunately, neither proper identification nor function of these bacteria has been established so far.

4. Benefits of Sponge-Bacteria Association

The putative benefits of bacteria to their sponge hosts include involvement in the nutritional process either by intracellular digestion or by translocation of metabolites (Wilkinson and Garrone, 1980; Borowitzka et al., 1988). Other possible functions of bacteria in the sponges include autotrophy, nitrogen fixation, nitrification (Wilkinson and Fay, 1979), stabilization of sponge skeleton (Wilkinson et al., 1981) and the participation in the host chemical defense system against predators and epibionts (Bakus et al., 1986; Paul, 1992; Proksch, 1994; Unson et al., 1994; Thakur et al., 2003). Because bacteria play a major role in microfilm formation, they become especially vital for soft bodied organisms like sponges to regulate bacterial epibiosis on their surfaces. Some recent studies have indicated that sponge associated bacteria play an important role in host epibacterial defense (Holmström et al., 1996; Holmström and Kjelleberg, 1999; Thakur and Anil, 2000; Thakur et al., 2003; Thakur et al., 2004). A benefit for the bacteria might be the mere protection ("hiding") by the sponge and subsequent escape from the grazing pressure in the environment. The bacteria also benefit from the stable food-rich environment that the sponge provides.

5. Importance in Drug Discovery

The biology of the sponge-bacteria association has elicited considerable interest among researchers investigating marine organisms as source of natural products. In recent years, marine sponges have been considered as a rich reserve of compounds possessing novel structures and biological

activities (reviewed in Faulkner, 1997; Schröder et al., 2003). However, because sponges harbor diverse bacterial populations, there is scientific debate about whether the sponge and/or its associated bacteria produce these molecules. There are some conflicts about the origin of the compounds in sponges; i.e., surfactin-like depsipeptides were originally isolated from a marine sponge. However, it has later been shown that the bacterium *Bacillus pumilus*, which is associated with the sponge, is the real producer of these compounds (Kalinovskaya et al., 1995).

Similar was the case with okadaic acid, a cytotoxic compound, which is now commercially available for biochemical research. Recently, our group has isolated a compound 2-methylthio-1,4-naphthoquinone from an unknown bacterium associated with the marine sponge *Dysidea avara*. This compound showed strong anti-angiogenic and antimicrobial properties (Müller et al., 2003b). A recent publication of Piel et al. (2004) also describes a biosynthesis of an antitumor polyketide by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei*. All these observations highlight the importance of sponge-bacteria associations not only for biodiversity research but also for biotechnology.

6. Molecular Biological Approach

It is widely accepted that culture-based techniques are inadequate to study bacterial diversity from environmental samples, because many bacteria can not be cultured by the current and traditional techniques. The use of molecular approaches for describing microbial diversity has greatly enhanced the knowledge of population structures in natural microbial communities. Combination of classical histological and microscopical techniques with new approaches in molecular biology has made the detection of bacterial symbiosis in sponges much easier.

To identify microorganisms in different marine organisms, including sponges, various culture-independent methods are now available. Cloning and sequencing of 16S rRNA genes give data that can be used to describe complete microbial community composition (Manz et al., 1992; Amann et al., 1995). It is also possible to infer nutritional requirements and physiological niches of many microbes based on information already available for known phylogenetic relatives. In combination with in situ hybridization techniques, the presence and abundance of a given isolate can be confirmed in its natural environment. Several recent studies have sought to address the phylogenetic diversity of microbial communities associated with marine sponges by using 16S rRNA gene sequence analysis (Preston et al., 1996; Althoff et al., 1998; Lopez et al., 1999; Hentschel et al., 2002; Fieseler et al., 2004). The rapid emergence of such techniques underlines the importance of molecular biological approach in the evaluation of sponge-bacteria symbiosis.

7. Sponge Model Systems

In this review, remarkable examples of the bacterial associations in marine sponges like *Suberites domuncula* and *Ircinia fusca* are discussed to show that this association could be an ideal model for symbiosis study.

Sponge Suberites domuncula

The marine sponge *Suberites domuncula* typically grows on snail shells. The snail shell can either be empty, a living shell occupied by snail, or a dead shell occupied by a hermit crab. These sponges occur in the sub-tidal region at 5–10 m depths. They are compact, slightly compressible, brightly colored and have a smooth waxy texture. Because of their association with hermit crabs they can live in habitats with sand or mud bottoms. The sponge *S. domuncula* has been found to have a close association with bacteria (Thakur et al., 2003). The bacteria were isolated from the sponge surface as well from the sponge primmorphs (3-D aggregates containing proliferating cells; Müller et al., 1999). Primary cell culture of the sponge *S. domuncula* was developed in the laboratory under sterile conditions. However, we did not succeed in eliminating some of the sponge associated bacteria. The presence of bacteria in the sponge and in primmorphs was traced by using bacterial rRNA, and the results showed that the bacterial population was present even after dissociation of sponge tissue.

As a further step, two bacterial strains were isolated and cultured from the primmorphs (Fig. 3), which displayed antibacterial activity. With 16S rRNA gene sequences, they showed 94 to 95% similarity with the genus *Pseudomonas* and they were not growing properly on normal Zobell agar plates after repeated subculture. It is conceivable that they require specific, possibly host-derived, factors for sustained growth that are missing in standard laboratory media. Interestingly, the construction of 16S rDNA libraries from

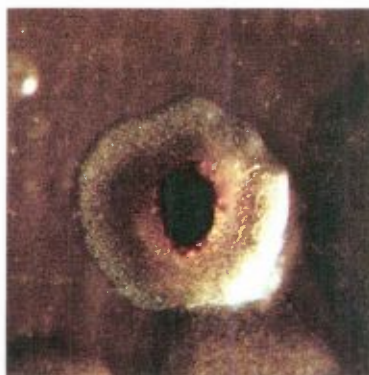


Figure 3. From seven days old sponge primmorphs (3-D aggregates containing proliferating cells), a bacterial strain was isolated and cultured. Primmorphs were observed to be surrounded with a bacterial colony, which could not grow on Zobell agar plates after repeated subculture, indicating close association with the sponge cells.

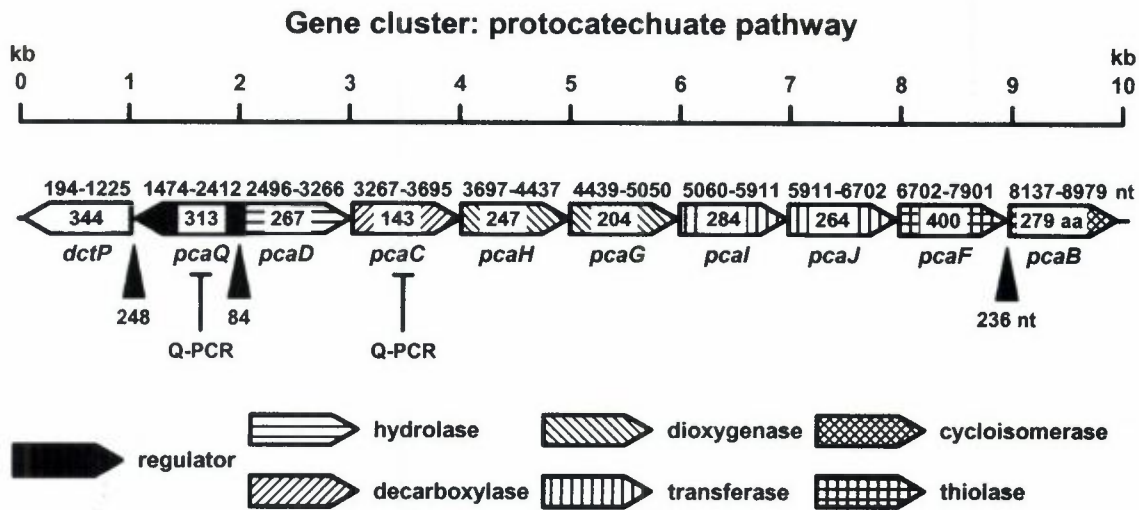


Figure 4. Structure of the gene cluster encoding the protocatechuate enzymes, which catabolize protocatechuate, isolated from the bacterium. The DNA segment is given above. Below, the borders of the genes are schematically presented; the numbering of the nucleotides refer to the ORF of the respective deduced polypeptide (the size, number of aa, is given within the boxes). The arrows indicate the direction of transcription. Some segments within the cluster were selected for quantitative PCR.

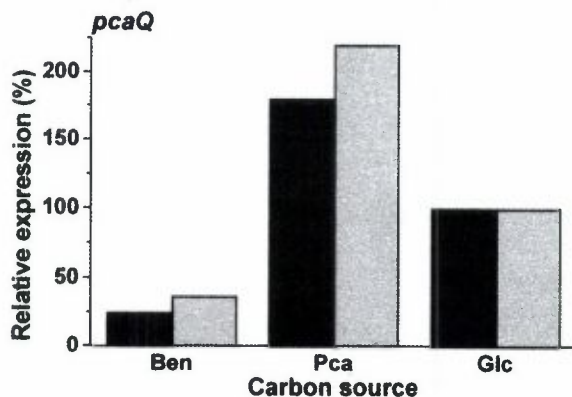


Figure 5. Expression level of the protocatechuate cluster in the sponge surface-associated bacterium SB2. The level of expression of the cluster was determined by quantitative PCR. RNA from bacterium, cultured in M9 minimal salt medium, supplemented with Na-benzoate (Ben), protocatechuate (Pca) or glucose (Glc). RNA was extracted, reverse transcribed and the resulting level of cDNA for the *pcaQ* genes from the protocatechuate cluster was determined. Two different dilutions of cDNA in the assays were analyzed; a 5-fold dilution (black bars) and a 25-fold dilution (gray). The level of expression is correlated with the level of the respective transcripts, measured in bacteria grown in the presence of glucose.

aquarium-maintained *S. domuncula* revealed only one sequence type of the genus *Pseudomonas* (Böhm et al., 2001). This study provides further evidence that *Pseudomonas* bacteria may be symbiotically associated with *S. domuncula* sponges.

The surface-associated bacteria were found to be involved in the indirect epibacterial defense of this sponge species. The association of some specific bacteria with their host

sponge (*S. domuncula*) was evaluated using a molecular biological approach. During this investigation, a bacterial strain SB2 (alpha-Proteobacterium MBIC 3368), which showed a specific association with this sponge species, was isolated from the sponge surface. This bacterium displayed antibacterial, cytotoxic, and anti-angiogenic potential (Thakur et al., 2005). The genome of this bacterial strain showed gene clusters of polyketide synthases, a class of enzymes that are involved in the biosynthesis of antibiotics and other bioactive metabolites (Saxena et al., 2003). The genome of this bacterium also showed a protocatechuate gene cluster (Fig. 4), which codes for the enzymes responsible for the conversion of protocatechuate to succinyl-CoA and acetyl-CoA (Buchan et al., 2000). The SB2 bacterium grew well in M9 minimal salt medium supplemented with the dihydroxylated aromatic compound protocatechuate; this carbon source supported its growth more than glucose as a carbon source (Fig. 5) (Müller et al., 2004).

Experiments were planned to find the source of diphenolic compounds for the growth of SB2 bacterium in its natural habitat. The genes for the enzymes tyrosinase (O₂ metabolizing) and δ -aminolevulinic acid dehydratase (ALAD; transporting), which convert monophenolic compounds into diphenols were isolated and cloned from the sponge *S. domuncula*. It was demonstrated that the metabolite protocatechuate, formed by the sponge tyrosinase/dioxygenase (Fig. 6), causes a strong up-regulation of the transcription of the protocatechuate cluster in the SB2 bacterium. The positive correlation between expression of the tyrosinase gene and the activity of tyrosinase with the abundance of this bacterium on the

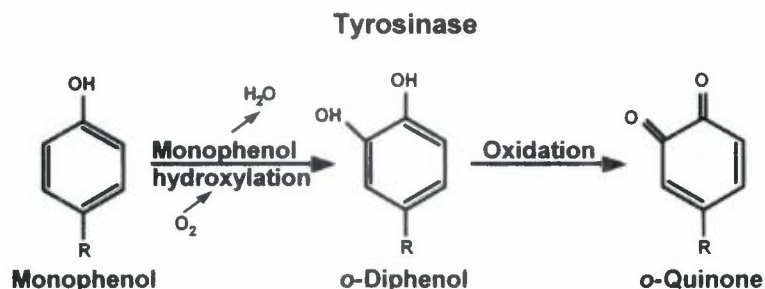


Figure 6. Formation of o-quinone (shown here) or protocatechuate by the sponge tyrosinase/ dioxygenase.

sponge surface can be considered as the first compelling experimental evidence of a sponge-bacteria symbiosis.

The principle of the symbiotic relationship, the production of diphenols via the tyrosinase in sponges and their (potential) transfer to the surface-associated bacteria will contribute to the further exploration of sponge-bacteria symbiosis. In return, SB2 was found to play an important role in the epibacterial defense of the host, by deterring fouling bacteria from the sponge vicinity. It is important to note that the SB2 bacterial strain (alpha-Proteobacterium MBIC 3368) has been isolated from different sponges irrespective of their taxonomic identity or geographical location (Webster et al., 2001; Hentschel et al., 2001). Further investigations with this bacterium are underway to highlight its symbiotic association with the sponge *S. domuncula*.

Sponge Ircinia fusca

The influence of environmental changes on the epibacterial population of the marine sponge *Ircinia fusca* (Carter) was evaluated by using a bacterial culture-based approach (Thakur et al., 2004). This sponge is of the class Demospongiae and grows like a wall (lamellar growth form) in the intertidal rocky habitats and, in spite of the severe competition for space by fouling organisms, it remains free of macro-epibionts. During five different collection periods, surface-associated bacteria from this sponge species were isolated, counted and cultured.

All culturable sponge-associated bacteria were studied morphologically and the isolates displaying bioactive potential were further identified using 16S rRNA gene sequences. It was found that the epibacterial population of the sponge is influenced by the temporal changes in the environment. However, the bacterial strain belonging to the genus *Bacillus*, which displayed antibacterial activity against fouling bacteria, was found to be associated with the sponge in all collection periods, indicating the close association of this strain with the sponge *I. fusca*. The sponge seems to have a selective control of the bacterial epibiosis on its surface. The antibacterial assay of sponge extract against its associated bacteria showed that the sponge promotes the growth of certain antibiotic producing strains of the genus *Bacillus* which may indirectly help the sponge

in governing the epibacterial population on its surface by deterring the settlement of other microbes from the vicinity.

This observation compares with the earlier reports that some bacterial strains are specific for the host, independent of any temporal and geographic variation (Webster and Hill, 2001). The culturable bacterial strain NW001 has shown such specificity with the marine sponge *Rhopaloeides odorabile*, collected from the Great Barrier Reef (Webster and Hill, 2001). An experiment carried out with the sponge *Aplysina aerophoba*, using molecular biological tools, has also shown that some bacteria permanently reside in the sponge mesohyl, indicating a close interaction between the host and associated bacteria (Friedrich et al., 2001).

Finally, the phylogeny of unicellular cyanobacterial symbionts in sponges from Australia and the Mediterranean Sea has been investigated by Usher and co-workers (2004). They found one symbiont, *Candidatus Synechococcus spongiorum*, in diverse sponge genera in the Mediterranean Sea and the Indian, Pacific, and Southern oceans. It has been proven that the maintenance of sponges in aquaria reduces the number of associated bacterial species. However, a few bacterial strains still remain encapsulated in special cells, the bacteriocytes (Böhm et al., 2001). Further investigations to explore the nature of association of bacterial strain of genus *Bacillus* with the marine sponge *Ircinia fusca* are in progress.

8. Conclusions and Perspectives

Sponge biologists are increasingly aware that the formation of alliances between sponges and bacteria is a widespread phenomenon. The present state of our knowledge on sponge-bacterial associations involves a variety of experimental approaches, with the culture of associated microorganisms from the sponges having special prominence. However, molecular biological approaches will help to greatly expand our understanding regarding the phylogenetic relationships between the bacteria and their sponge hosts. These techniques will be immensely valuable to explore sponge-bacteria symbiosis, particularly for unculturable bacterial assemblages. Our understanding of how bacteria interact with their hosts is still fragmentary and some general concepts involved in the symbiosis

process need to be addressed. These include avoidance of host defense systems, modulation of host cells, intracellular multiplication, and transmission of bacteria to new host cells. Several investigators have reported phylogenetically complex yet uniform microbial communities in marine sponges. However, individual sponge-bacterial interactions will be considered as commensalistic rather than symbiotic until a beneficial function for at least one partner has been demonstrated. Finally, investigations on the sponge-bacterial associations should lead to useful models for symbioses in marine invertebrates.

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