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Review article

Marine sponges as models for commensal microbe-host interactions

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Abstract

In nature, multiple-species rather than single-species microbial associations with plant or animal (including human) hosts are the rule more than the exception. Prominent examples are the microbial consortia of vertebrate intestines and cattle rumen. As many demosponges are associated with enormous amounts of microorganisms, contributing up to 40–60% of the sponge biomass, they are excellent models for marine multi-species, microbe-host associations. Representatives of at least eight different phyla, many of which contain few or no cultivated representatives, have been identified as specific members of the sponge-associated microbiota. Recent studies show that vertical transmission of symbionts through the larval stages rather than horizontal acquisition from seawater appears to be an important mechanism by which the complex and possibly ancient microbial consortia of sponges are formed.

Keywords: Sponge, Porifera, microbial consortia, symbionts, commensal, 16S rRNA

1. Introduction

Symbioses in the classical sense of de Bary (1879) are nowadays defined as mutualistic, commensal or pathogenic bacteria-host interactions (Douglas, 1994; Hentschel et al., 2000; 2002). Mutualistic bacteria-host interactions are balanced relationships with reciprocal benefit. Commensal interactions imply the coexistence of at least two different organisms without detriment, but possibly with a benefit to one partner. In pathogenic interactions, one partner benefits to the detriment of the other causing cell or tissue damage or even death of the organism. The interactions between bacteria and their hosts are not static, rather they evolve continuously to maintain equilibrium. While mutual symbioses are frequently ancient in time, pathogenic ones seem to have evolved more recently (Hentschel et al., 2000; Steinert et al., 2000).

Many of the well known symbioses come from the marine world (Table 1). They are typically based on primary metabolism where symbionts provide access to autotrophically fixed nutrients (carbon or nitrogen) in a nutrient-poor environment.

For example, through the acquisition of photoautotrophic symbionts, corals can build large reefs in tropical oligotrophic waters. On the other hand, a number of bacterial and fungal pathogens have been identified as the causative agents of coral bleaching (Rosenberg and Ben-Haim, 2002). Through incorporation of chemoautotrophic symbionts, worms, clams and mussels can populate deep-sea hydrothermal vents, cold seeps, seawage outfalls and anoxic sediments (Fischer, 1990). Through the aid of cellulose-digesting symbionts, ship-boring bivalves are able to establish an existence in driftwoods (Distel, 2003). Other marine symbioses are based on secondary metabolism where microbial symbionts provide chemical defenses for their hosts as it appears to be the case for bryozoans and possibly also for sponges (Haygood et al., 1999). An unusual behavioural symbiosis is represented by the light-producing *Vibrio fischeri* symbionts of sepiolid squids that probably serve to deter predators at night (Nyholm and McFall-Ngai, 2004). By providing essential functions (i.e., nutrient acquisition, chemical defense, behavioural mimicry) the respective animal hosts can populate new niches that would otherwise be inaccessible. This appears to be an important ecological advantage in the homogenous ecosystem of the ocean where physical barriers and geographic structures are scarce.

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Table 1. Selected marine symbiosis models.

Animal phylum	Invertebrate host	Microbial symbiont	Type of interaction	Proposed function of symbiont
Porifera	Sponges (i.e., *Aplysina aerophoba, Theonella swinhoei, Discodermia dissoluta, Rhopaloeides odorabile, Cymbastela concentrica*)	Sponge-specific lineages within the domains Bacteria [*Alpha-, Gamma-, Deltaproteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Gemmatimonadetes, Nitrospira* and *cand. phylum* Poribacteria] and Archaea (*Crenarchaeota*)	Commensal	Unknown
Cnidaria	Corals (i.e., *Acropora tenuis, Stylophora pistillata*) and sea anemonies (i.e., *Anthopleura elegantissima*)	Dinoflagellate algae (*Symbiodinium* sp.)	Beneficial	Photosynthetic symbionts provide CO_2 to the coral host
Cnidaria	Corals (various)	*Gammaproteobacteria* (*Vibrio* sp.)	Pathogenic	Symbiont expulsion and bleaching
Annelida	Oligochaetes (*Olavius* sp., *Inanidrilus* sp.)	*Gamma-* and *Deltaproteobacteria*	Beneficial	Chemoautotrophic symbionts provide host with multiple sources of nutrition and probably recycle worm waste products
Vestimentifera	Worms (*Riftia pachyptila*)	*Gammaproteobacteria*	Beneficial	Chemoautotrophic symbionts provide CO_2 to the gutless host
Mollusca	Shipworms (Teredinidae i.e., *Lyrodus pedicellatus, Bankia setacea, Xylophaga atlantica*)	*Gammaproteobacteria* (i.e., *Teredinibacter turnerae*)	Beneficial	Heterotrophic symbionts provide enzymes for cellulose digestion
Mollusca	Squids (*Euprymna scolopes*)	*Gammaproteobacteria* (*Vibrio fischeri*)	Beneficial	Bioluminescent symbionts provide light to deter predators at night
Mollusca	Sea slugs (i.e., *Elysia chlorotica*)	Heterokont algae (*Vaucheria litorea*)	Beneficial	Algae chloroplasts provide photoautotrophically fixed carbon to animal host
Mollusca	Bivalves and mussels (i.e., *Lucinoma* sp. *Bathymodiolus* sp.)	*Gammaproteobacteria*	Beneficial	Chemoautotrophic and methanotrophic symbionts provide CO_2 to the gutless host
Bryozoa	Moss animals (i.e., *Bugula neritina, B. simplex*)	*Gammaproteobacteria* (*Candidatus Endobugula glebosa*)	Beneficial	Heterotrophic symbionts provide secondary metabolites (polyketides) for chemical defense
Vertebrata	Fish (i.e., *Photoblepharon palpebratus* ('Eyelight fish')	*Gammaproteobacteria* (*Photobacterium* sp.)	Beneficial	Bioluminescent symbionts provide light to deter predators and attract prey and are used for communication with mates

Most of the established marine symbiosis models are based on the interaction of a given host or host lineage with one or few specific symbionts. Among the invertebrate hosts, certain oligochaetes are unusual in that they are specifically associated with at least four different symbiotic lineages. The metabolically different microorganisms presumably allow the worm to migrate through oxidizing and reducing sediments (Woyke et al., 2006). Among the

microbial symbionts, *Symbiodinium* algae are unusual in that a single symbiotic lineage (clade C) populates many dozens of different Great Barrier Reef corals (LaJeunesse et al., 2003).

In comparison to the well-studied symbioses mentioned above, the microbial associations of sponges are different in several respects: Rather than the known one (or few)-symbiont-one-host types of associations, the microbial consortia of sponges are exceedingly complex containing sponge-specific representatives of at least eight different prokaryotic (bacterial and archaeal) phyla. To our knowledge, no other animal phylum tolerates such amounts of internal, freely dispersed microorganisms. Most other symbionts are housed in specific, symbiont-bearing cell layers (gastrodermis of corals and sea anemonies) or organs (gland of Deshayes in shipworms, light organs of squids, trophosome tissue of vestimentiferan worms) or even in specialized cells, termed bacteriocytes. Because sponges are sufficiently distinct from the other established marine symbioses, they are worthwhile models to study marine commensal bacteria-host interactions.

2. The Players: Sponges as Animal Hosts

The phylum sponges (Porifera) forms one of the deepest radiations of the Metazoa with a fossil record dating back more than 580 million years (Li et al., 1998). Well over 1,000 sponge fossils have been described within 15 genera and 30 species in Precambrian rock deposits suggesting an early radiation of this phylum. The phylum Porifera is divided into three classes, the Calcarea (calcareous sponges), the Hexactinellida (glass sponges) and the Demospongiae, which contain more than 90% of the sponges living today. An estimated 13,000 living sponge species are found on tropical reefs but also with increasing latitudes and even in freshwater lakes and streams. Sponges are known for their colorful appearance and the morphological plasticity that ranges from encrusting layers several millimeters in height to massive tubular sponges of >1 meter in size.

Sponges are diploblast metazoans that lack true tissues or organs. In spite of their simple organisation, genome sequencing has revealed genes that are highly similar to those of vertebrates (Müller et al., 2001). As sessile filter-feeders they pump large volumes of water through a specialized canal system, termed the aquiferous system. The filtration capacities of sponges are remarkably efficient amounting to many thousands of liters kg^{-1} day^{-1}. While typical seawater contains 10^5–10^6 bacteria ml^{-1}, the seawater expelled from the sponge is essentially sterile (Wehrl et al., in press). Food particles such as unicellular algae and bacteria are retained in the choanocyte chambers and translocated into the mesohyl interior where they are rapidly digested. The mesohyl serves as a scaffold that is made up of extracellular matrix and constitutes much of the sponge body. Single, amoeboid sponge cells, termed archaeocytes, move freely through the mesohyl matrix and digest food particles by phagocytosis.

3. The Players: Microbial Consortia as Symbionts

Many species of demosponges are known to contain large amounts of bacteria within their tissues, which may contribute up to 40–60% of the tissue volume (for reviews, see Lee et al., 2001; Hill, 2004; Hentschel et al., 2006). These sponges are called 'bacteriosponges' or 'high microbial abundance sponges' (Vacelet and Donadey, 1977; Wilkinson, 1978; Hentschel et al., 2003b; Schmitt et al., submitted). Microbial concentrations amount to 10^8 to 10^{10} microbial cells ml^{-1} sponge extract exceeding the concentrations of seawater by two to five orders of magnitude. The vast majority of microorganisms are located extracellularly within the mesohyl matrix. Microorganisms are also found within vacuoles of archaeocytes where they appear in various stages of digestion. In some cases, microorganisms are located within the nuclei of certain sponge cells. The sponge surfaces, the canal system and choanocyte chambers are noticeably free of microorganisms. There are other sponge species that coexist in the same habitat whose mesohyl is essentially free of microorganisms. They are referred to as 'low microbial abundance sponges' (Vacelet and Donadey, 1977; Wilkinson, 1978; Hentschel et al., 2003b; Schmitt et al., submitted). The microbiota of 'low microbial abundance sponges' reflects that of seawater, both in numbers and in phylogenetic composition. The reasons why some sponges are hosts to large intrinsic microbiota while others are virtually devoid of microorganisms are currently unknown.

About a dozen studies have addressed the phylogenetic diversity of microbial communities associated with marine bacteriosponges using 16S rRNA gene library construction and other 16S rRNA gene based techniques (i.e., Webster et al., 2001b; Hentschel et al., 2002; Schirmer et al., 2005; Taylor et al., 2005). The sponges of these studies are taxonomically only distantly related, have geographically non-overlapping distribution patterns and contain host-specific secondary metabolite profiles. In spite of these differences, the sponges contain a uniform microbial signature (Hentschel et al., 2002). Particularly representatives of the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* (formerly CFB group or *Cytophagales*), *Chloroflexi* (formerly green-non-sulfur bacteria), *Cyanobacteria*, *Proteobacteria* (*Alpha-*, *Gamma-*, *Delta-*) and *Nitrospira* were frequently recovered by this approach (Fig. 1). The Harbor Branch Marine Microbial Database (HBMMD)(http://www.hboi.edu/dbmr/dbmr_hbmmd.html) represents an extensive collection of culturable

microorganisms from marine sponges and other deep-water invertebrates (Gunasekera et al., 2005). However, none of the above-mentioned, sponge-specific bacteria have been cultivated so far with one noticeable exception. The alphaproteobacterial strain MBIC3368 has been isolated from at least eight different sponge species (Scheuermayer et al., 2006) including adult and larvae samples of the sponge *Mycale laxissima* (Enticknap et al., 2006).

Archaea have been identified in the sponge *Axinella mexicana* (Preston et al., 1996; Schleper et al., 1998). Originally described as *Cenarchaeum symbiosum*, close relatives have since been found in the Australian sponge *Rhopaloeides odorabile*, in Mediterranean sponges of the genus *Axinella*, and in eight different sponge species from the Korean coast (Lee et al., 2003; Margot et al., 2002; Webster et al., 2001a). Interestingly, representatives of this

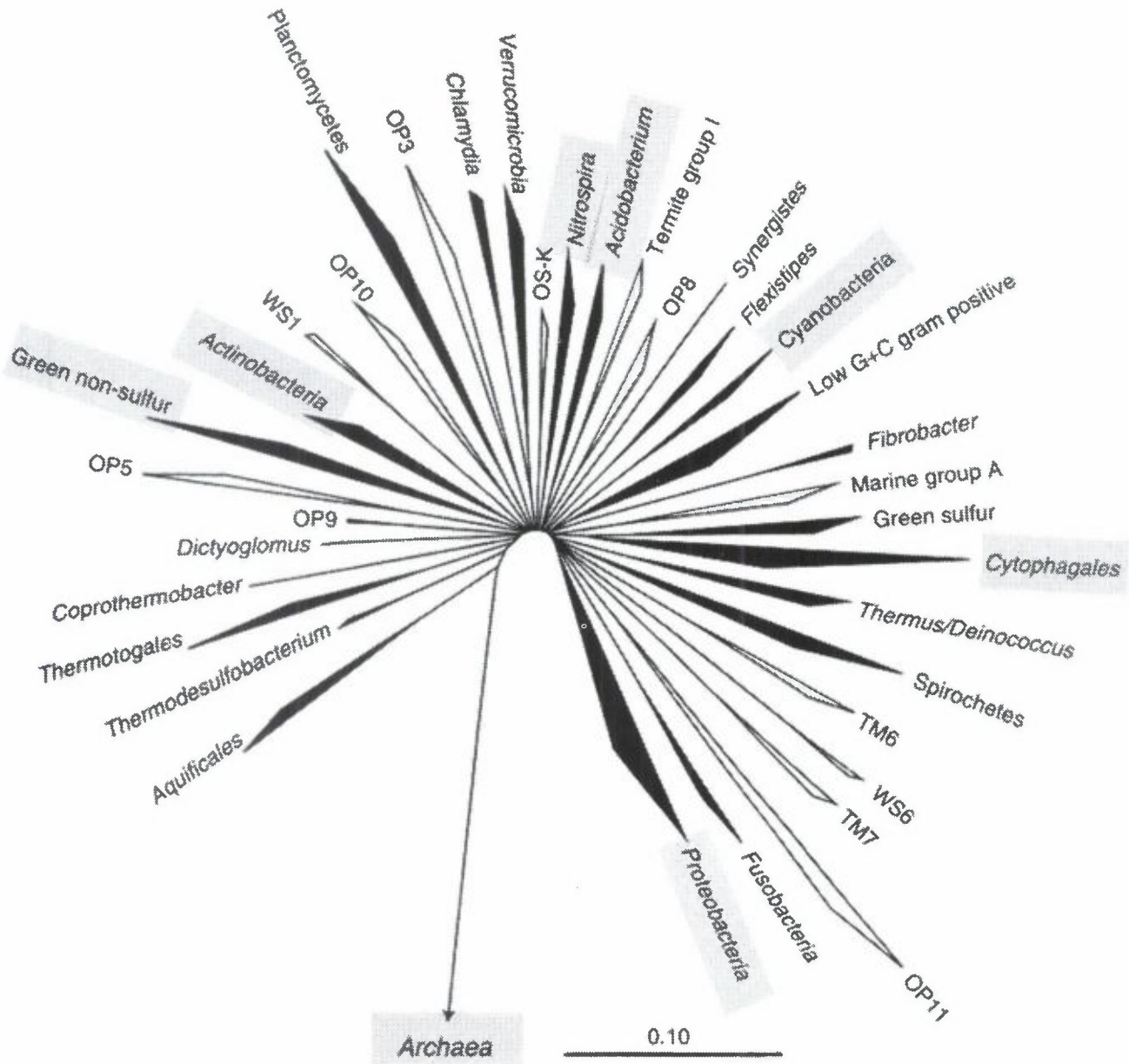

Figure 1. Phylogenetic tree of the prokaryotes modified after Hugenholtz et al. (1998). Phyla, of which members of the sponge-specific microbiota were identified are marked with grey boxes. The *Candidatus* phylum 'Poribacteria' falls into the *Planctomycetes-Verrucomicrobia-Chlamydiae* superphylum (Wagner and Horn, 2006). The green non sulfur bacteria are now termed *Chloroflexi*. Most lineages were also identified by DGGE in the reproductive stages of *I. felix* with the exception of the *Nitrospira* and *Cyanobacteria* (Schmitt et al., in press).

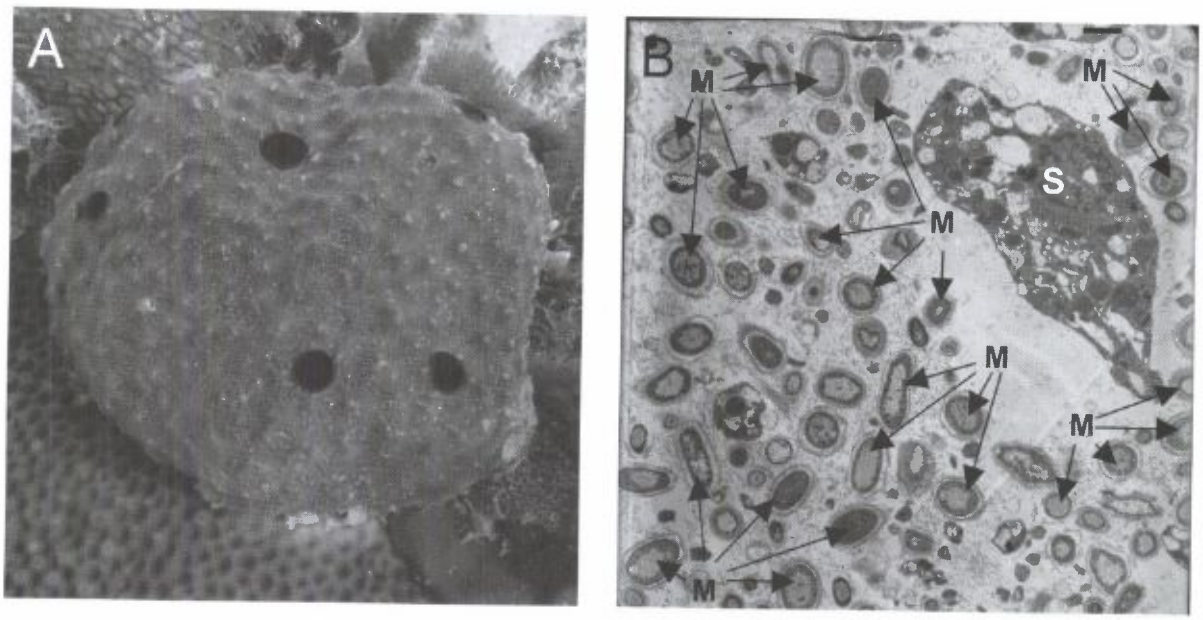

Figure 2. (A) The bacteriosponge *Ircinia felix* (Irciniidae) from the Florida Keys, USA. (B) Transmission electron micrograph of *I. felix* larvae collected from the water column. M: Microorganisms, S: Sponge cell. Scale bar: 1 µm. Underwater picture and electron micrograph by S. Schmitt.

clade have been obtained in pure culture from a seawater aquarium and were shown to be autotrophic and able to oxidize ammonium to nitrite (Könneke et al., 2005). It therefore appears likely that archaeal symbionts use ammonium, which is excreted by sponges as a metabolic end product, as an energy source.

In addition, a novel *Candidatus* phylum 'Poribacteria' has recently been discovered in various marine sponges. The 'Poribacteria' are widely distributed in marine high microbial abundance sponges but have not been found in seawater or sediments. They are characterized by the presence of a nucleoid-containing organelle (Fieseler et al., 2004). Nucleoid-containing bacterial morphotypes were identified in sponges by electron microscopy in early studies (Vacelet and Donadey, 1977), and more recently by Fuerst and co-workers (1999) who also documented the presence of DNA in the nucleoid-like compartments. The morphological diversity of these unusual bacteria encompasses short and long rod-shaped cells as well as D-shaped cells with typical gram-negative cell walls. S-layer structures and bleb-like extrusions of the outer membrane were also noted. Metagenomic library construction and sequencing of a 39 kb Poribacteria-positive fosmid clone showed that the 16S rRNA gene is unlinked from a conventional *rrn* operon (Fieseler et al., 2006). Additionally, a novel kind of molybdenum containing oxidoreductase as well as a series of eight ORFs encoding for unusual transporters and channel or pore forming proteins were identified. This environmental genomics approach provided, for the first time, genomic and, by inference, functional information on the so far uncultivated, sponge-associated candidate division 'Poribacteria'.

4. Horizontal Acquisition of Symbionts from Seawater

Because of the massive filtration rates of sponges, the hypothesis that the microbial symbionts are acquired from seawater was tested. Feeding experiments with six taxonomically different bacterial isolates were performed and the uptake rates were determined over time by plating seawater aliquots to determine the number of colony forming units in the incubation water (Wehrl et al., in press). Furthermore, feeding experiments were performed with enriched microbial seawater consortia and sponge symbiont consortia that had been obtained by physical separation following established protocols (Fieseler et al., 2006). Because the sponge symbiont consortia and seawater consortia contain a large fraction of uncultivated microorganisms, their uptake rates were determined by DAPI-counting. The uptake rates of the pure cultures and the microbial seawater consortia were very efficient and fell in the same high range (up to 2.76×10^6 bacteria g^{-1} sponge wet weight $hour^{-1}$). In contrast, the uptake of sponge symbiont consortia was significantly reduced by almost two orders of magnitude. The internal processing of ingested particles and bacteria in the mesohyl of *A. aerophoba* was documented microscopically. While the GFP-labelled *Vibrio* sp. was immediately digested upon contact with the mesohyl, fluorescent latex beads were taken up efficiently

and appeared in the mesohyl in concentric rings. Rhodamine-live-labelled symbionts did not enter the mesohyl at all (Wehrl, 2006). While the reasons for the apparent resistance of the symbionts to uptake and digestion remain unknown, these results provided no evidence for horizontal uptake of symbionts from seawater. However, symbiont acquisition from seawater may still occur in selected instances or by chance and therefore, this hypothesis should not be discarded entirely for the lack of experimental data.

5. Vertical Transmission of Symbionts through the Reproductive Stages

Vertical transmission has been documented in marine bryozoans (Haygood and Davidson, 1997; Lim and Haygood, 2004) and bivalves (Cary and Giovannoni, 1993). Since microorganisms had been visualized in the reproductive stages of various sponges by electron microscopy (Ereskowsky et al., 2005 and references cited therein) the hypothesis was followed whether symbionts are transmitted vertically in the sponge *Ircinia felix* (Schmitt et al., in press) and several other species (Schmitt et al., submitted) using a combination of electronmicroscopical and molecular techniques.

I. felix is a common, ball-shaped sponge of the Caribbean (Fig. 2A) that releases parenchymella-type larvae in synchronized spawning events. Electron-microscopical inspection revealed large amounts of morphologically diverse microorganisms in the adult and in the center of larvae that were collected singly from the water column (Fig. 2B). In *I. felix* juveniles, bacteria were found in between densely packed sponge cells. Denaturing gradient gel electrophoresis (DGGE) revealed highly similar microbial profiles in the adult and its reproductive stages. In total, over 200 bands were excised and sequenced. The phylogenetic diversity of adult *I. felix* and its larvae was equally high and included members of the phyla *Alpha-*, *Gamma-* and *Deltaproteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, *Bacteroidetes* and a clade of uncertain taxonomic affiliation. 63% of the adult, 54% of the larval and 26% of the juvenile sequences were composed of members of the sponge-specific microbial community described previously (Hentschel et al., 2002).

In summary, it could be shown that phylogenetically complex microbial consortia are transmitted vertically to the next *I. felix* sponge generation. These results are consistent with Sharp et al. (2007) who reported on the consistent presence of three bacterial lineages throughout the embryonic development of the tropical sponge, *Corticium* sp.. Moreover, Enticknap et al. (2006) showed vertical transmission of culturable, sponge-specific *Alphaproteobacteria* through the larvae of *Mycale laxissima*. These cumulative results help to gain a better understanding of the evolution and ecology of this possibly very ancient sponge-bacteria association.

6. Conclusions

In comparison to the many pathogenic and symbiotic types of interactions, the commensal ones have traditionally been the most difficult to study. However, the understanding of commensal microbial consortia is of high importance because bacteria rarely exist as monocultures in nature. It is well known that complex microbial consortia play important roles in various ecological contexts (i.e., human intestine, cattle rumen, marine sponges) and it is becoming increasingly clear that they also have an immediate effect on the nutrition, immune system and the development of their invertebrate and vertebrate hosts (Hooper and Gordon, 2001; Hentschel et al., 2003a). With the implementation of molecular tools for microbial community analysis, it is now possible to define the members of the microbial communities (culturable and non-culturable), and to analyze their functions in the symbiosis context, as has been shown for marine sponges in this review. Studies of this kind will lead to a more comprehensive understanding of commensal bacteria-host interactions that goes well beyond the one-bacterium-one-host concept.

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