

Review Article

## Significance of Microbial Symbionts in Sponge Evolution and Ecology

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### Abstract

Marine sponges contain a wide variety of microbial symbionts, often with many different symbionts in the same species. Some bacterial symbionts appear to have evolved with the hosts since Precambrian times, and the symbiosis has continued because masking capsules have obscured the symbionts from sponge phagocytic cells. Cyanobacterial symbionts are important for many tropical and warm temperate sponges through the addition of carbon energy compounds and fixed nitrogen to the symbiosis. The role of bacterial symbionts is obscure. Symbiont bacteria are capable of metabolizing a wide range of organic compounds, thereby adding to the nutrition of the host/symbiont system. In addition, there is an unexplained direct relationship between the size and density of sponges and the content of bacterial symbionts which can occupy up to 40% to the tissue volume. Both symbiont types may have aided sponge evolution by permitting the hosts to cope with increasing levels of atmospheric oxygen. Marine sponges may prove to be ideal models for studying the role of microbial symbioses in Metazoan evolution.

Keywords: symbiosis, marine, sponges, bacteria, cyanobacteria, zooxanthellae, ecology, evolution

## 1. Introduction

The driving force behind the evolution of the eukaryotic cell was the invasion of some bacterial 'hosts' by other microorganisms which eventually lead to stable symbioses. Following approximately 2600 million years with only prokaryotes on the earth, the first eukaryotic cell appeared about 1200 million years ago. The first metazoans appeared in the fossil record about 650 million years ago (Olson, 1981) and sponges are likely candidates to be amongst those first metazoans because of the 'primitive' type of organization and the close similarities between sponge cells and many protozoans.

The hypothesis, that the eukaryotic cell evolved via microbial invasion of other prokaryotes resulting in the incorporation of these microbes as organelles, has gained strong acceptance (Margulis, 1970). Little attention, however, has been paid to the possible role of microbial symbionts in the evolution of the Metazoa. Modern sponges contain a wide variety of prokaryotic and eukaryotic symbionts, with some of these appearing to have been with their hosts since the late Precambrian. A study of these symbioses may provide clues to the evolution of higher metazoans. In this paper the nature and possible roles of microbial symbionts in sponges will be reviewed as a basis for a speculative discussion on the role of these symbionts in the evolution and ecology of sponges.

## 2. Microbial Symbionts

### *Bacteria*

These are frequently observed as a granulation in the intercellular mesohyl of marine sponges, but bacterial symbionts do not occur in freshwater species. Electron microscope observations of marine sponges reveal as many as 7 and possibly more, morphologically different bacteria as symbionts within a sponge (Vacelet and Donadey, 1977; Wilkinson, 1978a). The proportion of bacterial symbionts varies considerably from barely detectable populations to dense assemblages occupying as much as 40% of the sponge tissue volume (Bertrand and Vacelet, 1971; Wilkinson, 1978a). Bacterial symbionts are reported to occur in all extant marine sponges (Wilkinson, 1984), although they are sparse in the Hexactinellida (Mackie and Singla, 1983). Some of these symbionts, and possibly all, are specific to sponges and not found in the ambient water (Wilkinson et al., 1981). One specific symbiont was found in all sponge species examined from the Classes Demospongiae, Calcarea and Sclerospongiae but not in the Hexactinellida (Wilkinson, 1984). This led to speculation that this symbiont became associated with evolving sponges in

the Precambrian, after the separation of these Classes from the hexactinellids. This distinct separation is supported by Reiswig and Mackie (1983) who consider that the hexactinellids should be regarded as a subphylum of the Porifera.

The presence of bacteria adjacent to phagocytic cells within sponges which are potent consumers of bacteria from the ambient water (Reiswig, 1971, 1973; Wilkinson, 1978b), presents an enigma as to how symbiont bacteria resist being digested. Wilkinson et al. (1984) found that when sponge-specific bacteria were fed back to host sponges, they passed through uneaten. Therefore it was hypothesized that the bacterial symbionts escaped recognition through the possession of masking capsules. Moulder (1979) described the intracellular environment as a particularly harsh one for potential microbial symbionts and recorded that there is rarely more than one type of symbiont per cell. The mesohyl of a sponge is comparable to an intracellular environment in that it is totally bounded by cellular tissue and contains the full variety of sponge cells, most of which are phagocytic. Therefore, the second enigma is why there are many different symbionts within the same, potentially hazardous, environment.

### *Cyanobacteria*

At least 4 different cyanobacteria occur as symbionts both intracellularly and intercellularly within marine sponges (this number may expand as other sponges are examined). Two unicellular symbionts are described as species of *Aphanocapsa* (Sara, 1971) although they are unlike free-living species of the genus; a multicellular form has been designated as *Oscillatoria spongelliae* (Sara, 1966); another unicellular form is yet to be named, but it resembles *Synechocystis trididemni* (Rützler, 1981).

One of the unicellular species (cf. *A. feldmanni*) is by far the most widely distributed, occurring in at least 14 sponge Orders of the Classes Calcarea and Demospongiae (Wilkinson, 1980a), particularly in tropical sponges (Wilkinson, 1983a; 1987a; Wilkinson and Trott, 1985). Cyanobacterial symbionts are only recorded in sponges from warm temperature to tropical habitats and only those growing in relatively shallow water (<30 m depth). The host species may be divided into two categories on the basis of the number of symbionts and the contribution made to the sponge. Most species have a thin layer of tissue containing the cyanobacteria in those parts exposed to light, whereas some flattened sponges contain numerous symbionts distributed throughout the tissue. The latter species are termed phototrophic

because cyanobacteria make a major contribution to host nutrition, while in the former, there is little contribution (Wilkinson and Trott, 1985).

#### *Unicellular Eukaryotes*

The most prominent are dinoflagellate symbionts within boring clionid sponges (Pang, 1973). The source of these symbionts is unknown. However, it appears more than coincidental that the symbiosis appears restricted to the boring sponges which frequently bore into living zooxanthellate corals. Recently a diatom has been observed in symbiosis with one sponge species (Cox and Larkum, 1983). Many freshwater sponges contain *Chlorella* symbionts which contribute to the nutrition of the hosts by translocating glucose (Wilkinson, 1980b). There are a number of other symbioses in sponges which involve multicellular algae (Vacelet, 1982).

### 3. Bacterial Symbioses in Evolution and Ecology

Sponges in the fossil record can be traced back to the late Precambrian ( $650 \times 10^6$  y B.P.) with three existing sponge Classes already evident (Finks, 1970; Ziegler and Rietschel, 1970). The finding of immunologically similar bacteria in all species of three sponge classes in widely separate geographical locations suggested that the symbiosis commenced as a single event prior to taxonomic diversification, rather than as many later events (Wilkinson, 1984). Morphologically-comparable cyanobacteria are found over a similar wide spectrum of sponge species, therefore the same reasoning may be applicable: the cyanobacterial symbioses commenced prior to the bulk of sponge speciation. The limited range of hosts for the dinoflagellate and chlorellae symbionts may be indicative of more recent acquisition e.g. freshwater sponges evolved from marine sponges which lost all bacterial symbionts (Wilkinson, 1984) when they invaded freshwater habitats within the last 50 million years (Bergquist, 1978) and took up chlorellae from the ambient waters.

The evolution of sponges, probably from a coalition of different protozoa (Hadzi, 1963), occurred during an elevation of the oxygen atmosphere from 0.2% ( $1200 \times 10^6$  y B.P.) to levels approaching 20% (Olson, 1981). Sponges today are essentially aerobic animals but they contain a range of bacteria from strict anaerobes (Imhoff and Truper, 1976), through facultative anaerobes to aerobes (Wilkinson et al., 1981). Such bacteria have the ability to metabolize an extensive range of organic compounds under almost any atmospheric conditions (Wilkinson, 1978c; Wilkinson et al., 1981). This association would enable host sponges to grow in a wide range of habitats using

almost all organic matter, provided there was sufficient exchange of nutrients between the partners. Bacterial symbionts have been shown to incorporate amino acids more rapidly than host cells (Wilkinson and Garrone, 1980) and presumptive nutrient translocation has been demonstrated from cyanobacterial symbionts in some sponges (Wilkinson, 1979). The contribution by the cyanobacteria was confirmed when sponges in the light grew faster than those in the dark (Wilkinson and Vacelet, 1979).

The largest bacterial populations, up to 40% of tissue volume, occur in large, dense sponges where it is apparent that the size of the population is proportional to the density of the sponge tissue (Wilkinson, 1978b). Most bacterial symbionts produce extracellular capsules (Vacelet, 1975; Vacelet and Donadey, 1977; Wilkinson, 1978a) and these would contribute to the augmented density. Whether this increase density affects sponge size is unknown, however, freshwater sponges without bacterial symbionts (Wilkinson, 1984) never attain the sizes of marine species.

The demonstration that some marine sponges contain green and purple photosynthetic bacteria (Imhoff and Truper, 1976) suggests that the host sponges may have been able to exist in near anaerobic conditions during their evolution and may do so today. It is possible that some sponges derive photosynthate from these symbionts, which are sequestered under conditions of low oxygen tension in the dense mesohyl. The metabolism of reduced sulphur compounds, alluded to in Imhoff and Truper (1976), would provide another source of energy rich compounds. Anaerobic chemotrophic symbionts are crucial to the growth of other marine invertebrates living adjacent to deep hydrothermal vents (Jannasch, 1985) or in organically rich, anaerobic muds (Cavanaugh, 1983).

A role in the rearrangement of skeletal sponging fibres has been suggested for one of the specific bacterial symbionts which possesses the enzymes necessary to digest the collagen, whereas sponge cells are incapable of digesting collagen (Wilkinson et al., 1979).

#### 4. Algal Symbioses in Evolution and Ecology

Cyanobacterial symbionts are important for the survival and growth of many sponges in clear water environments and it is possible that they played the same role in some sponges throughout their evolution. Sponges and corals alternated as the dominant organisms in reef formation throughout the Palaeozoic and early Mesozoic (Ziegler and Rietschel, 1970) and one explanation for this alternative domination may lie in the nature and suitability

of the photosynthetic symbionts during the various periods of reef growth. Currently, corals dominate reef formation through the production of structurally sound but not dense skeletons, with assistance from the zooxanthellae. Modern survivors of the reef-building sponges, by contrast, produce very dense skeletons (Schuhmacher and Plewka, 1981) and do not contain photosynthetic symbionts. Extant sclerosponges are only found in cryptic habitats, apparently unable to compete with the faster growing corals. It is unknown whether the earlier, massive sponges contained cyanobacteria which may have contributed to the competition with the coral ancestors, however, near relatives (Vacelet, 1983) do not contain such symbionts (Wilkinson, 1980a).

The current role of symbiotic cyanobacteria is more readily comprehended. These symbionts have been shown to be essential for the survival of a number of sponge species (Wilkinson and Vacelet, 1979) many of which appear to be dependent on the symbionts for much of the carbon energy requirements (Wilkinson, 1983b, 1987b). Possession of these symbionts permits sponges to occupy habitats of low ambient nutrient concentration but with high levels of incident light; these areas abound on shallow continental shelves of tropical and subtropical seas. On the Great Barrier Reef, phototrophic sponges (dependent on photosynthetic symbionts) constitute approximately 50% of the total sponge biomass in clean water habitats whereas they are virtually absent from higher productivity, more turbid waters (Wilkinson and Trott, 1985). In contrast, phototrophic sponges are virtually absent on Caribbean reefs although approximately 40% of sponge species have a low concentration of cyanobacterial symbionts (Wilkinson, 1987a).

Phototrophy in sponges may be a recent phenomenon as only 12 species of approximately 300 found in the central part of the Great Barrier Reef are dependent on the symbionts for the greater part of the nutrition, whereas at least 50% of the species contain at least some cyanobacterial symbionts. These 12 species plus 2 others from the Red Sea (Wilkinson, 1979) are found across 4 Orders, indicating possible convergent evolution of host sponges which originally contained only a few symbionts. Phototrophic species are only reported to be prominent on reefs of the western Pacific and into the Indian Ocean as far as the Red Sea. Only one sponge in the Caribbean, *Chondrilla nucula*, was shown to be phototrophic with cyanobacteria but there are several other boring species with dinoflagellates which also appear to be phototrophic (Corredor et al., in press). These sponges constitute an insignificant proportion of the total sponge biomass on Caribbean coral reefs (Wilkinson, 1987a). As this region is considered depauperate with respect

to some reef species and has been subjected to considerable climatic and sea level changes (Vermeij, 1978), it is conceivable that either phototrophic species have become extinct or have not evolved in this more localized region.

In addition to fixing carbon, some marine sponges with cyanobacteria are able to fix atmospheric nitrogen (Wilkinson and Fay, 1979). This would aid sponge growth in regions of low ambient nitrogen availability, such as coral reefs.

A role in shielding underlying tissue from the harmful effects of UV radiation (Jokiel, 1980) has been suggested (Sara, 1971). Hermatypic corals produce UV blocking compounds (Dunlap and Chalker, 1986), however, no mechanism for UV protection in sponges has been reported.

Dinoflagellates in sponges have not been studied in detail. Most boring sponge species with these symbionts occur in well illuminated environments and one Caribbean species (*Anthosigmella varians*) has been shown to be phototrophic with an instantaneous P/R ratio in saturating light of 1.5 (Corredor et al., in press). Therefore, it seems probable that energy obtained through the symbiotic association with zooxanthellae may be used to decalcify and bore into corals, which use similar symbionts to aid in calcification.

## 5. Conclusions

It is hypothesized that many of the bacterial and cyanobacterial symbionts currently found in marine sponges became associated with the hosts during the early stages of Metazoan evolution in the late Precambrian. Some of these may have been associated with the early colonial protozoans, such as Protospongia/Proterospongia (Lackey, 1959; Saville-Kent, 1881), and have remained in symbiosis because masking capsules prevented recognition as food matter. Some of these symbionts, particularly the cyanobacteria, currently play an important role in sponge ecology, permitting the hosts to exploit illuminated habitats with low levels of available organic matter. A more important role may have been played by microbial symbionts in the evolution of sponges by permitting the hosts to occupy habitats of increasing oxygen concentration.

## REFERENCES

- Bergquist, P.R. 1978. *Sponges*. Hutchinson University Library, London.

- Bertrand, J.C. and Vacelet, J. 1971. L'association entre éponges cornées et bactéries. *C.R. Hebd. Séanc. Acad. Sci. Paris* **273**: 638-641.
- Cavanaugh, C.M. 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphide-rich habitats. *Nature* **302**: 58-61.
- Corredor, J.E., Wilkinson, C.R., Vicente, V.P., Morell, J.M., and Otero, E. 1987. Nitrate release by Caribbean reef sponges. *Limnol. Oceanogr.* (in press).
- Cox, G.C. and Larkum, A.W.D. 1983. A diatom apparently living in symbiosis with a sponge. *Bull. Mar. Sci.* **33**: 943-945.
- Dunlap, W.C. and Chalker, B.E. 1986. Identification and quantitation of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. *Coral Reefs* **5**: 155-159.
- Finks, R.H. 1970. The evolution and ecologic history of sponges during Palaeozoic times. *Symp. Zool. Soc. Lond.* **25**: 3-22.
- Hadzi, J. 1963. *The Evolution of the Metazoa*. Pergamon Press, Oxford.
- Imhoff, J.F. and Truper, H.G. 1976. Marine sponges as habitats of anaerobic phototrophic bacteria. *Microb. Ecol.* **3**: 1-9.
- Jannasch, H.W. 1985. The chemosynthetic support of life and the microbial diversity at deep-sea hydrothermal vents. *Proc. Royal Soc. London. B* **225**: 277-297.
- Jokiel, P.L. 1980. Solar ultraviolet radiation and coral reef epifauna. *Science* **207**: 1069-1071.
- Lackey, J.B. 1959. Morphology and biology of a species of Protospongia. *Trans. Amer. Micr. Soc.* **78**: 202-206.
- Mackie, G.O. and Singla, C.L. 1983. Studies on hexactinellid sponges. I. Histology of *Rhabdocalyptus dawsoni* (Lambe, 1873). *Phil. Trans. Royal Soc. London. B* **301**: 365-400.
- Margulis, L. 1970. *Origin of Eukaryotic Cells*. Yale University Press, New Haven.
- Moulder, J.W. 1979. The cell as an extreme environment. *Proc. Royal Soc. London. B* **204**: 199-210.
- Olson, J.M. 1981. Evolution of photosynthetic and respiratory prokaryotes and organelles. *Ann. N.Y. Acad. Sci.* **361**: 8-19.
- Pang, R.K. 1973. The ecology of some Jamaican excavating sponges. *Bull. Mar. Sci.* **23**: 227-243.

- Reiswig, H.M. 1971. Particle feeding in natural populations of three marine demosponges. *Biol. Bull.* **141**: 568-591.
- Reiswig, H.M. 1973. Bacteria as food for temperate-water sponges. *Can. J. Zool.* **53**: 582-589.
- Reiswig, H.M. and Mackie, J.O. 1983. Studies on hexactinellid sponges. III. The taxonomic status of the Hexactinellida within the Porifera. *Phil. Trans. Royal Soc. London. B.* **301**: 419-428.
- Rützler, K. 1981. An unusual bluegreen alga symbiotic with two new species of *Ulosa* (Porifera: Hymeniacidonidae) from Carrie Bow Cay, Belize. *P.S.Z.N.I. Mar. Ecol.* **2**: 35-50.
- Sara, M. 1966. Associazioni tra Poriferi e alghe in acque superficiali del litorale marino. *Ricerca. Scient. Roma* **36**: 277-282.
- Sara, M. 1971. Ultrastructural aspects of the symbiosis between two species of the genus *Aphanocapsa* (Cyanophyceae) and *Ircinia variabilis* (Demospongiae). *Mar. Biol.* **11**: 214-221.
- Saville-Kent, W. 1881. *A Manual of the Infusoria*. David Bogue, London.
- Schuhmacher, H. and Plewka, M. 1981. Mechanical resistance of reefbuilders through time. *Oecologia* **49**: 279-282.
- Vacelet, J. 1975. Etude en microscopie électronique de l'association entre bactéries et spongiaires du genre *Verongia* (Dictyoceratida). *J. Microscopie Biol. Cell.* **23**: 271-288.
- Vacelt, J. 1982. Algal-sponge symbioses in the coral reefs of New Caledonia: a morphological study. *Proc. 4th Int. Coral Reef Symp.* **2**: 713-719.
- Vacelet, J. 1983. Les éponges calcifiées et les récifs anciens. *Pour la Science* **June**: 14-22.
- Vacelet, J. and Donadey, C. 1977. Electron microscope study of the association between some sponges and bacteria. *J. Exp. Mar. Biol. Ecol.* **30**: 301-314.
- Vermeij, G.J. 1978. *Biogeography and Adaptation Patterns of Marine Life*. Harvard University Press, Cambridge.
- Wilkinson, C.R. 1978a. Microbial associations in sponges. III. Ultrastructure of the *in situ* associations in coral reef sponges. *Mar. Biol.* **49**: 177-185.
- Wilkinson, C.R. 1978b. Microbial associations in sponges. I. Ecology, physiology and microbial populations of coral reef sponges. *Mar. Biol.* **49**: 161-167.

- Wilkinson, C.R. 1978c. Microbial associations in sponges. II. Numerical analysis of sponge and water bacterial populations. *Mar. Biol.* **49**: 169–176.
- Wilkinson, C.R. 1979. Nutrient translocation from symbiotic cyanobacteria to coral reef sponges. *C.R. Coll. Int. C.N.R.S. Paris* **291**: 373–380.
- Wilkinson, C.R. 1980a. Cyanobacteria symbiotic in marine sponges. In: *Endocytobiology Endosymbiosis and Cell Biology*. W. Schwemmler and H.E.A. Schenk, eds. Walter de Gruyter, Berlin, pp. 553–563.
- Wilkinson, C.R. 1980b. Nutrient translocation from green algal symbionts to the freshwater sponge *Ephydatia fluviatilis*. *Hydrobiologia* **75**: 241–250.
- Wilkinson, C.R. 1983a. Phylogeny of bacterial and cyanobacterial symbionts in marine sponges. In: *Endocytobiology*. Vol. II. W. Schwemmler and H.E.A. Schenk, eds. Walter de Gruyter, Berlin, pp. 993–1002.
- Wilkinson, C.R. 1983b. Net primary productivity in coral reef sponges. *Science* **219**: 410–412.
- Wilkinson, C.R. 1984. Immunological evidence for the Precambrian origin of bacterial symbioses in marine sponges. *Proc. Royal Soc. London. B.* **220**: 509–517.
- Wilkinson, C.R. 1987a. Interocean differences in size and nutrition of coral reef sponge populations. *Science* **236**: 1654–1657.
- Wilkinson, C.R. 1987b. Productivity and abundance of large sponge populations on Flinders Reef flats, Coral Sea. *Coral Reefs* **5**: 183–188.
- Wilkinson, C.R. and Fay, P. 1979. Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. *Nature* **279**: 527–529.
- Wilkinson, C. and Garrone, R. 1980. Nutrition of marine sponges. Involvement of symbiotic bacteria in the uptake of dissolved carbon. In: *Nutrition in the Lower Metazoa*. D.C. Smith and Y. Tiffon, eds. Pergamon Press, Oxford, pp. 157–161.
- Wilkinson, C.R., Garrone, R., and Herbage, D. 1979. Sponge collagen degradation *in vitro* by sponge-specific bacteria. *C.R. Coll. Int. C.N.R.S. Paris* **291**: 361–364.
- Wilkinson, C.R., Garrone, R., and Vacelet, J. 1984. Marine sponges discriminate between food bacteria and bacterial symbionts: electron microscope radioautography and *in situ* evidence. *Proc. Royal Soc. London. B.* **220**: 519–528.

- Wilkinson, C.R., Nowak, M., Austin, B., and Colwell, R.R. 1981. Specificity of bacterial symbionts in Mediterranean and Great Barrier Reef sponges. *Microb. Ecol.* **7**: 13-21.
- Wilkinson, C.R. and Trott, L.A. 1985. Light as a factor determining the distribution of sponges across the central Great Barrier Reef. *Proc. 5th Int. Coral Reef Cong.* **5**: 125-130.
- Wilkinson, C.R. and Vacelet, J. 1979. Transplantation of marine sponges to different conditions of light and current. *J. Exp. Mar. Biol. Ecol.* **37**: 91-104.
- Ziegler, B. and Rietschel, S. 1970. Phylogenetic relationships of fossil calcisponges. *Symp. Zool. Soc. Lond.* **25**: 23-40.