Hydrobiologia **461:** 1–7, 2001.
J.D. McKenzie (ed.), Microbial Aquatic Symbioses: from Phylogeny to Biotechnology.

Photosymbiotic associations in planktonic foraminifera and radiolaria

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Key words: sarcodine, photosymbiosis, molecular phylogeny, srDNA

Abstract

Foraminifera, radiolaria and acantharia are relatively large (>1 mm in most cases) unicellular eukaryotes that occur in pelagic oceanic communities. Commonly referred to as planktonic sarcodines, these organisms often harbor algal symbionts. The symbionts have been described as dinoflagellates, chrysophytes and prasinophytes based upon their morphology either in the host or as free-living organisms in culture. To investigate the molecular taxonomic affiliations of the algae, and to determine the sequence variability between symbionts from individual hosts, we examined the small subunit ribosomal DNA sequences from symbionts isolated from planktonic foraminifera and radiolaria. The symbionts that we analyzed included dinoflagellates, prasinophytes and prymnesiophytes.

We have, through our studies of planktonic sarcodine symbioses, and through comparison with other symbiotic associations (corals and lichens), observed that taxonomically distinct lineages of symbiotic algae are not uncommon. How do such different algae share the function of symbiosis, while other, more related algae, do not? We propose that there are commonalties that exist between symbiotic algae that confer symbiotic 'competence', and the way to begin the search for these is to utilize the different algal symbiont lineages.

Introduction

Various types of algae occur as intracellular symbionts in the pelagic protists commonly referred to as planktonic sarcodines (Fig 1). Members of the sarcodines include the foraminifera, the radiolaria and the acantharia. These ameboid marine protists form conspicuous biological assemblages, especially in oligotrophic oceans, and contribute significantly to local primary production via their algal symbionts. Sarcodines are additionally important components of epipelagic communities due to their predation upon algae and other planktonic organisms (Swanberg & Caron, 1991; Caron et al., 1995).

Photosymbioses among planktonic sarcodines have been known for well over a century, yet identification of the symbionts has often been hindered by the loss of diagnostic morphological features, such as flagella, thecae or scales, when the algae are in the symbiotic state. Studies of the free-living forms are useful, but the establishment of algal symbiont cultures can be difficult, and there is always a potential for obtaining non-symbiotic algae (contaminants). Many symbiont 'identifications' have, therefore, been limited to assignment to algal classes based upon ultrastructural features, such as plastid shape or nuclear structure. These latter characterizations are valuable, but they are typically insufficient to resolve taxo-

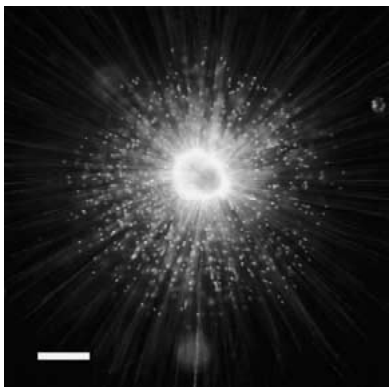

Figure 1. Juvenile *Orbulina universa*. Dinoflagellate symbionts (*Gymnodinium beii*) are the smaller dots distributed along the spines. Scale bar is ~200 μm.

nomic affiliations of symbionts among the various host species.

Researchers have described dinoflagellate, prasinophyte and chrysophycophyte (includes chrysophytes, bacillariophytes and prymnesiophytes) sarcodine symbionts based upon both ultrastructural features and/or culture (Anderson, 1983; Spero, 1987; Faber et al., 1988). Most of these descriptions have been controversial or ambiguous. For example, in 1993 Banaszak (Banaszak et al., 1993) proposed identification of the dinoflagellate symbionts cultured from *Velella velella* as *Scrippsiella* due to morphologic similarities. They also noted that the ultrastructural characteristics of the symbionts in the colonial radiolarian, *Collosphaera*, were very similar to those of the *Velella* symbionts. These radiolarian dinoflagellates were described by Brandt as *Zooxanthella nutricula* (Brandt, 1881), and have been moved to the genera *Amphidinium* and *Endodinium* in the intervening years (reviewed by Blank & Trench, 1986 and in Banaszak et al., 1993). Culture methods were also employed by Spero to identify the dinoflagellate symbiont of the planktonic foraminiferan, *Orbulina universa* (Spero, 1987). He placed the symbiont in the genus *Gymnodinium*, as opposed to its previous description as *Aureodinium* (Spindler & Hemleben, 1980, pp. 133–140). Prasinophyte and chrysophycophyte symbionts, in contrast to their dinoflagellate counterparts, have not been described from cultured (free-living) specimens and their identifications are therefore vague. Researchers have predicted that the green prasinophyte symbionts of the solitary radiolarian, *Spongodrymus*, would be closely related to the marine flatworm symbiont, *Tetraselmis* (Anderson, 1983, pp 121). The two mutually exclusive symbionts of *Globigerinella siphonifera* were distinguished and described as a chrysophyte and a prymnesiophyte (Faber et al., 1988; Gastrich, 1988).

These various analyses and descriptions have not provided a clear indication of the phylogenetic placement of the symbiotic algae in reference to other algal symbionts, nor an estimation of the species diversity among symbiont populations in different hosts and in different oceans. To further assess the taxonomic relationships of the algal symbionts in planktonic sarcodines, we utilized the analysis of small subunit ribosomal RNA gene sequences. In this paper, we will review the results of these sequence analyses, compare what we have learned about sarcodine photosymbioses with those in corals and lichens, and explore the concept of symbiotic lineages within algal taxa.

Materials and methods

Foraminifera and radiolaria were collected from several locations in the Sargasso Sea, 3–5 miles southeast of Bermuda, during September 1994 and May 1995. Sarcodines were collected individually by divers, to ensure that the cells were in good condition and to limit the clumping of organisms and association with debris (i.e. potential sources of contamination). *Velella velella* was collected from the Sargasso Sea in May 1995.

Individual organisms were transferred through three sterile seawater washes prior to microdissection of the symbiotic algae. Each microdissection was considered a single sample, and samples of dissected symbionts were not pooled. Several hundred symbionts were obtained from each individual host, with half used for nucleic acid extraction and half used to start symbiotic algal cultures. Microdissected algal samples were used to obtain initial small subunit ribosomal DNA (srDNA) sequence information and to confirm free-living algal culture identities. Dinoflagellate symbionts were isolated from five different planktonic foraminifera and six different colonial and solitary radiolaria. Three individuals of *Spongodrymus* and three individuals of *Globigerinella siphonifera* yielded other types algal symbionts.

Polymerase chain reaction amplification of the nuclear small subunit ribosomal RNA gene was accomplished using eukaryote-specific primers (for further details see Gast & Caron, 1996 and Gast et al., 2000). PCR products were digested with restriction enzymes to (1) determine if the symbionts from different hosts were similar, (2) to establish that free-living cultures were correct, and (3) to examine whether the dinoflagellate symbionts were related to any of the *Symbiodinium* RFLP groups described by Rowan (Rowan & Powers, 1991, 1992: Rowan & Knowlton, 1995).

PCR products for sequencing were obtained by pooling duplicate PCR reactions. Our goal was to reduce the effect of PCR errors and microdiversity within the original sample upon the final sequence. Direct sequencing of the products was accomplished using ^{35}S dATP and DynaBeads (Dynal) or infrared dye labeled primers (LI-COR). All of the symbiont sequences are available from GenBank (accession numbers: U37365, U37366, U37367, U37406, U41085, U41086, U41087, U52352-U52357, U52911, AF166376-AF166381).

Alignments were generated in GDE (Steve Smith, University of Illinois) with other algal sequences that were retrieved from GenBank. These sequences were chosen based upon either predicted taxonomic affiliations of the symbionts or identification of similarity through Blast (Bilofsky & Burks, 1988) searches using the symbiont sequences. Regions with questionable or unreliable alignment were excluded from the analyses. Phylogenetic reconstructions were accomplished using PHYLIP (3.4, Felsenstein, 1989), PAUP 3.0 and PAUP (4.0.0d64, David Swofford). The maximum likelihood tree for the dinoflagellates was generated using PHYLIP with the default parameters and the random addition of taxa. Bootstrap values for this dataset were obtained using 500 replicates of maximum parsimony heuristic searches with the random addition of taxa (PHYLIP). Maximum likelihood analyses for the non-dinoflagellate symbionts were accomplished using the default parameters in PAUP 4.0.0d64, with the random addition of sequences and TBR branch swapping. Bootstrap values were obtained from 1000 replicates of maximum parsimony heuristic searches with tree bisection-reconnection branch swapping and 10 random sequence additions per replicate. In all cases the trees were unrooted, but an outgroup was specified. (For further information on the datasets please refer to Gast & Caron, 1996 and Gast et al., 2000.)

Results

RFLP analysis

Three restriction enzymes (*Hinf* I, *Hae* III, *Taq* I) were used to distinguish between the symbionts isolated from planktonic foraminifera and radiolaria. The sarcodine dinoflagellate symbionts were unique, with regard to each other and to *Symbiodinium* (Gast & Caron, 1996). The RFLP analyses also allowed us to determine that the dinoflagellate symbionts from *Velella* were potentially very closely related to the symbionts from the radiolaria, and sequence analysis confirmed that the two symbionts were almost identical (Gast & Caron, *op. cit.*).

We also used the RFLP patterns to confirm that our free-living symbionts were the same as those originally microdissected and not a contaminant recovered through culture. Shown in Figure 2 is an example of RFLPs using all three enzymes on the full-length PCR products from the symbionts of *Globigerinella siphonifera*.

Tree summaries

The maximum likelihood trees for the dinoflagellate and the non-dinoflagellate symbionts of the planktonic sarcodines are shown in Figure 3. Bootstrap values at the nodes are from maximum parsimony analyses. In our previous work (Gast & Caron, 1996), we found that the foraminifera dinoflagellate symbiont (*Gymnodinium beii*) was closely related to *Symbiodinium*, and to the free-living species, *Gymnodinium simplex*. In contrast, the radiolarian dinoflagellate symbiont (*Scrippsiella nutricula*) and the dinoflagellate symbiont from *Velella* (*Scrippsiella velellae*) were not similar to any of the other symbiotic dinoflagellates identified at this point (other symbiont taxa indicated with a star).

The non-dinoflagellate symbionts that we examined from planktonic sarcodines were identified as prasinophytes and prymnesiophytes. The prediction that the prasinophyte symbionts isolated from the solitary radiolarian, *Spongodrymus*, were related to *Tetraselmis* was confirmed. Calculations of percent base differences for sequences within the clade of *Scherffelia* and *Tetraselmis* indicated that our symbionts were equally distinct from both genera, and probably represent a separate, but related, genus. *Globigerinella siphonifera*, a planktonic foraminiferan, hosted the prymnesiophyte symbiont. The closest taxon currently available in the database was *Prymnesium*, but it represents only a distant relative. These prymnesiophyte symbionts correspond to ones identified in TEM studies as 'Type I' (Faber et al., 1988). (For further information on the free-living morphology of this symbiont see Gast et al., 2000.) The second symbiont type of *G. siphonifera* is described as a chrysophycophyte or perhaps a chrysophyte (Faber et al., 1988). Samples of this symbiont type have not yet been subjected to molecular analysis.

Note: Very recently the srDNA sequence for *Chrysochromulina acantha* was made available in GenBank. This organism is now the closest relative of our Type I prymnesiophte symbiont, with 11 base differences between the two sequences.

Diversity within symbiont taxa

We found very little 'within group' variation in the srDNA from each of the symbiont types that we studied. Differences between *Scrippsiella velellae* and *Scrippsiella nutricula* were 0.2%, or 4 bases out of approximately 1800. This low level of nucleotide vari-

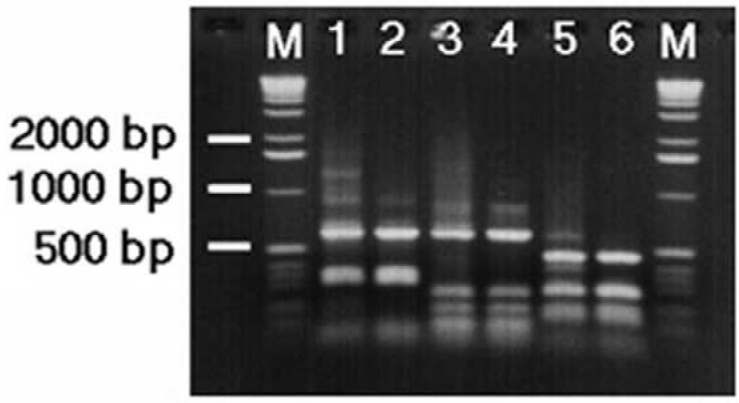

Figure 2. Restriction digest of PCR products from symbionts of *Globigerinella siphonifera*. Agarose gel stained with ethidium bromide. Lanes 1, 3, and 5 are *Hae* III, *Hinf* I and *Taq* I digests of microdissected symbiont PCR products, respectively. Lanes 2, 4 and 6 are *Hae* III, *Hinf* I and *Taq* I digest of cultured symbiont PCR products. m = marker; 1 kilobase-pair ladder (Gibco/BRL).

ability was also the case within the prasinophyte and the prymnesiophyte samples.

The most sequence variation that we observed occurred within the *G. beii* lineage. Base differences in the srDNA sequences of symbionts isolated from different *Orbulina universa* ranged from 0 to 14 bases, with the typical amount being 6 bases. This variation is similar to (or less than) that seen within the different *Symbiodinium* groups (A, B and C). In our work with ribosomal sequences, we have considered nucleotide variation less than 1% to represent organisms of the same species. While this is an arbitrary definition on our part, we believe that it has served us well in our examination of sarcodine symbionts. The symbionts within each symbiont 'type' (e.g. *G. beii*) could be considered strains of the same species.

Discussion

Comparison with lichen and coral photosymbioses

We have summarized in Table 1 some of our observations comparing planktonic sarcodine symbioses with those of corals and lichens. In this comparison, we have split the planktonic sarcodines into the foraminifera and the radiolaria since they are taxonomically unrelated. One of the shared characteristics initially noted was the presence of a primary algal symbiont. We regard a primary algal symbiont as one that has been identified in more than half of the relationships examined. Planktonic foraminifera and radiolaria exhibit a situation where there is a single sequence type that represents the primary symbiont. We could define this as a single species based upon the molecular information, but we are hesitant to do so because we know very little about the physiological traits of these symbionts. The corals and the lichens show more ribosomal sequence diversity in their primary symbiont. Despite these differences, the coral symbionts still form a coherent genus, *Symbiodinium*, and it may eventually become appropriate to refer to the different *Symbiodinium* sequence groups as species. *Trebouxia* has recently been the target of ribosomal sequencing, and this data confirms the genetic similarity of different isolates of the same algal species, as well as the differences between species (Friedl & Rokitta, 1997; Beck et al., 1998). This species/strain sequence diversity may have arisen in the older host/symbiont relationships and perhaps represents greater divergence or diversity of physiological function. The single sequence type for the planktonic sarcodines may also change as more associations from different locations are studied.

Lichens, radiolaria and foraminifera are also capable of forming symbioses with other algal taxa. Besides the symbionts that we have examined molecularly, the foraminifera are also thought to harbor chrysophytes, and the radiolaria, a very small green alga. Lichens are perhaps the most extreme in that they include cyanobacterial symbionts in their suite of potential symbionts, in addition to chlorophyte algae (Douglas, 1994).

It is very interesting that all these relationships show some degree of host/symbiont flexibility. Over the past several years, it has been observed that host/symbiont relationships appear to be less strict than was traditionally thought. The lack of molecu-

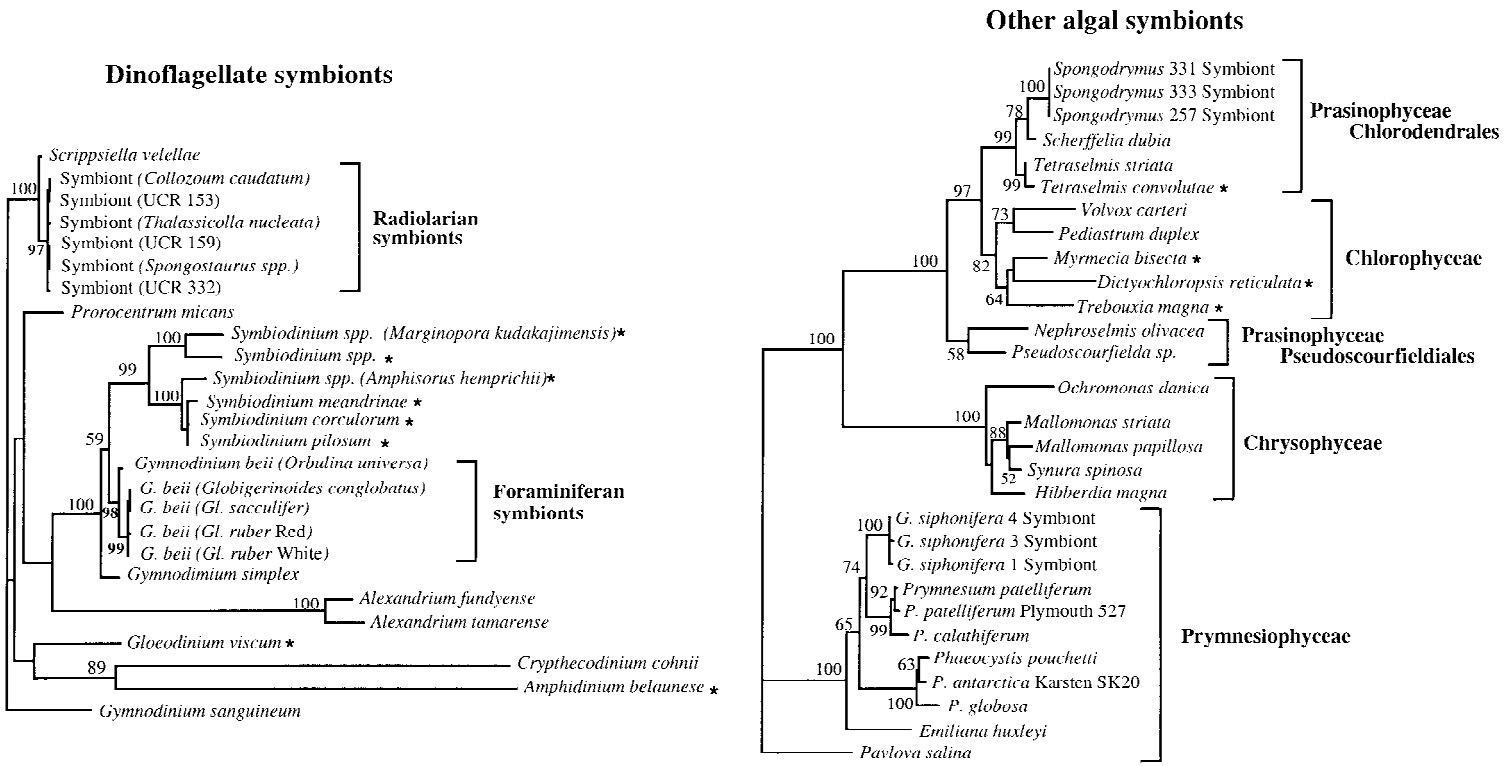

Figure 3. Phylogenetic reconstructions (maximum likelihood and bootstrapped maximum parsimony) for dinoflagellate and non-dinoflagellate symbionts involved in planktonic sarcodine symbioses. An outgroup taxon was used for each reconstruction, but the trees are unrooted. Bootstrap values below 50% are not shown. Symbiont sequences from organisms other than the planktonic sarcodines are marked with asterisks. *Amphisorus* and *Marginopora* are benthic foraminifera.

Table 1. Comparison of features involved in photosymbioses of planktonic sarcodines, corals and lichens

Photosymbiotic association	Primary algal symbiont	Other algal taxa as symbionts	Same host with different symbiont	Different host with same symbiont
Planktonic Foraminifera	*Gymnodinium beii* Dinophyceae	Yes Chlorophyceae, Prymnesiophyceae	No	Yes (within the forams)
Radiolaria (Colonial and Solitary)	*Scrippsiella nutricula* Dinophyceae	Yes Prasinophyceae	Yes	Yes
Coral	*Symbiodinium* spp. Dinophyceae	No	Yes (at the level of the symbiont species)	Yes
Lichen	*Trebouxia* spp. Chlorophyceae	Yes Cyanobacteria, other Chlorophyceae	Yes	Yes (within the lichens)

larly identifiable co-evolution between many hosts and symbionts has been the impetus for this argument. The same (or similar) host can have different symbionts, or different hosts can harbor the same symbiont. Both situations are illustrated by the dinoflagellate and prasinophyte symbionts that are present in the same genus of solitary radiolarian (*Spongodrymus*), as well as in pelagic chondrophores and marine flatworms, respectively. In the lichens, *Chaenotheca* is an example of a single host that is able to harbor any of four green algal genera as a symbiont (Ahmadjian, 1993).

Planktonic foraminifera, corals and lichens also provide examples of divergent hosts having similar symbionts. Planktonic foraminifera have ribosomal gene sequences that are quite distinct (Darling et al., 1997), yet most of them possess *G. beii* as a symbiont (Gast & Caron, 1996). Molecularly similar isolates of *Symbiodinium* are also shared between corals, bivalves, anemones and jellyfish (Carlos et al., 1999 and reviewed in Rowan, 1998). Within the lichens the same species of *Trebouxia* are also shared by different hosts (Ahmadjian, 1993). Recent molecular analyses have confirmed the genetic similarity of isolates of the same species of *Trebouxia*, but they also indicate that the genus may not be monophyletic (Friedl & Rokitta, 1997; Beck et al., 1998).

Coral symbioses are of further interest due to presence of more than one symbiont type. Rowan & Knowlton (1995) reported that individual coral heads of *Montastrea* could be populated by two different *Symbiodinium* types at the same time (Rowan & Knowlton, 1995). Data on other types of corals have suggested that the presence of multiple symbionts is not unusual, but perhaps overlooked by previous studies (Rowan, 1998). We have not observed more than one symbiont type at a time in the planktonic sarcodines. The situation appears to be different for the benthic foraminifera (Lee et al., 1985).

We do not suggest that symbiotic interactions are random, but are instead selective. Often the algae in marine photosymbioses are not vertically transmitted, and the juvenile organisms must acquire the symbiont from the mixed bag of symbiotic and non-symbiotic algae in the water column. A specific symbiont may be preferable, but at some point it may be more important for the host to simply have a functional symbiont. How the host and symbiont recognize each other, establish and maintain the relationship remains a mystery. The flexibility of many of these interactions suggests a common mechanism for the general recognition of symbionts with the subsequent selection of a specific alga.

Symbiont lineages

We have noted several taxonomically distinct symbiont lineages in our study of sarcodine photosymbioses. Similarly, studies of coral and lichen pho-

tosymbioses, have noted the presence of unique symbiotic lineages in the eukaryotic algae (dinoflagellates, prasinophytes, prymnesiophytes, chlorophytes and chrysophytes) as well as the prokaryotes (cyanobacteria). Some of these lineages currently represent a single species or strain (*Gymnodinium beii*) whereas others appear to be a collection of related species (*Symbiodinium* and *Trebouxia*). These lineages can be viewed either as a surprising diversity of organisms, or as a relatively small number given the multitude of algal taxa in the world. We prefer the first, but in either case the fact that symbioses have evolved many times is clear. It is also certain that as we continue to look, we will find more of these independent symbiotic lineages within the algae.

What do these multiple lineages, and the flexibility of these host-symbiont associations, mean in the larger sense of photosymbiosis? In this regard, we have begun to speculate about what makes an alga a suitable symbiont. These associations are considered highly evolved, yet we see highly distinct algae (taxonomically) serving apparently equally well as photobionts, while very similar are often not acceptable. Presumably there is some common physiological ability (or inability) shared by these algae that confers symbiotic competence. Only through the study of divergent algal lineages can we hope to identify these commonalties. (WHOI contribution # 10091)

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