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Initial studies of dinoflagellate symbiont recognition in Soritinae

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Abstract

All of the diatoms that are endosymbiotic within larger foraminifera (Phylum Granuloreticulosea, Protista) share in common a 104 kDa glycoprotein on their surfaces which is not found on the surfaces of diatoms which are not symbiotic. This common symbiont surface antigen (CSSA) is recognized by host granuloreticulopodia and is necessary for the establishment and maintenance of the symbiotic diatoms within their hosts. There is reason to believe that a different group of symbiont-bearing foraminifera Soritinae also recognize epitopes on the surfaces of the endosymbiotic dinoflagellates they host. We tested polyvalent antisera containing antibodies (including the CSSA) raised against the frustule fractions of three taxonomically diverse species of diatoms (*Nanofrustulum shiloi*, *Nitzschia panduriformis*, *Amphora tenerimma*) against *Symbiodinium* spp isolated from soritine foraminifera and from *Casseopea frondosa*. There was no reaction to the antisera suggesting that a different surface antigen(s) or some other system must be involved in signaling between these dinoflagellates and their foraminiferan hosts.

Keywords: Antibodies against endosymbiotic diatoms, Common Symbiont Surface Antigen (CSSA), endosymbiotic dinoflagellates, *Symbiodinium*, *Nanofrustulum shiloi*, *Nitzschia panduriformis*, *Amphora tenerimma*, Soritinae, larger foraminifera

1. Introduction

A phyletic diversity of microalgae are involved in endosymbioses with invertebrates and protists. The fact that most of the known symbioses restrict certain hosts to particular species of algae or types of algae (reviewed by Trench, 1993) has to suggest that there are mechanisms for hosts and symbionts to recognize each other and establish long-lived or stable specific relationships (Trench, 1988). There seems to be a consensus that the specificity observed in naturally collected specimens is the result of a multi-step process that begins with initial recognition, proceeds to establishment, and ends with a protracted maintenance of the relationship (Trench, 1993). Two opposing hypotheses on the concept of selectivity have credence. One, advanced by Rahat and Reich (1988) based on their studies of *Chlorella* and *Hydra* suggests that the algae are "preadapted" to persist in certain host cells and that selectivity is based on cell *milieu*. Even if the symbionts find themselves in phagosomes, which are converted to phagolysosomal vesicles, the algae avoid digestion, or cellular destruction, by some mechanism or another.

The example often given in Microbiology classes is catalase production by species of *Staphylococcus* which prevents them from being oxidized by H_2O_2 released in host's phagolysosomes. An alternate hypothesis is based on ligand-receptor interactions and various signal transduction events. In this line of thinking there are specific molecules (ligands) on the cell walls or surfaces of algal symbionts that interact with receptors on the uptake surfaces of animals or protists. This initial contact signal leads to an altered state of the phagosome (to a symbiosome, Jeon, 1997) and prevents phagosomes from being converted to phagolysosomal vacuoles.

Studies of diatom endosymbiosis in a number of families of larger foraminifera were initially quite puzzling. The same species of foraminifera was the host for any one of score of a variety of different diatom species, many of which, belong to different diatom families (reviewed by Lee, 1998). The list of diatom species was not endless and after several thousand examinations of host foraminifera it was clear that six species of diatoms were involved in roughly 75% of all the symbioses. What did the symbiotic diatoms have in common that species of diatoms that were captured and digested, as food did not have? Immunological studies showed that endosymbiotic diatoms

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shared in common a 104 kDa glycoprotein on their surfaces that was not found on the surfaces of diatoms that served as food and were digested (Chai and Lee, 1999a,b). With the aid of immunogold labeled preparations for the TEM, receptors were found for this Common Symbiont Surface Antigen (CSSA) on the reticulopodia of the host foraminifera. Blocking the antigen or receptors interfered with both the recognition and establishment processes (Chai and Lee, 2000).

Isolation of the dinoflagellates endosymbiotic in the larger foraminiferan subfamily Soritinae piqued our curiosity. Is it possible that the soritine-*Symbiodinium* spp symbioses share common antigens and a common signaling system with the diatom-bearing foraminifera?

The first question to ask is whether there is any evidence that a signaling system may be present in the dinoflagellate-soritine symbioses? One very intriguing piece of evidence was a report by Polne-Fuller (1991) who found that the marine amoeba *Trichosphaerium* ate many different kinds of algae but strangely did not digest any strain of *Symbiodinium* that she tested. She suggested that the amoebae might be useful as tools to obtain axenic cultures of *Symbiodinium*. Perhaps significant in this respect is the report of Markell et al. (1992) who extracted the amphaesmas of *Symbiodinium microadriaticum*, *S. kawagutii* and *S. pilosum*. They found polypeptides ranging in mass from 14–200 kDa. However, they did not find any obvious correlation in the SDS-PAGE patterns from the different species examined. They (Markell et al., 1992; Markel and Trench, 1993) also looked at the exudates from symbiont cells grown in culture after 30 days. They found that in batch culture the algae exude (40–50 pg cell^{-1}) a mixture of proteins, neutral sugars, sugar amines and uronic acids. They also found in infection experiments with *C. xamachana* all three of these species of *Symbiodinium* exuded more uronic acids than those strains that did not infect. Consistent with their SDS-PAGE results, antibodies (anti-SmXuL) produced against the large molecular fraction (>50 kDa) of polysaccharides from *S. microadriaticum*, did not cross react with those of the other species tested. Trench (1993) commented on some unreported TEM studies that he and Markell had done that showed anti-SmXuL labeling in hospite of a select group of host cell nuclei. He also noted that "in the process of transport from the algae to the host amoebocytes and then to the host tissues there was no evidence of vesiculation of the glycoconjugates."

It is with the above background in mind that we began the experiments reported here.

2. Materials and Methods

Isolation and culture of Symbiodinium *from Soritids*

Isolation of *Symbiodinium* from soritines was not an easy task. The hosts have surfaces that are similar in architecture to houses with tiled roofs. There are many crevices for epiflora to attach. Vigorous brushing and digestion of the tests (shells) helps reduce this source of contamination. In addition, some soritid hosts have secondary symbionts (verified in histological preparations, Lee et al. 1997). The methodology for isolation has been published (Lee et al., 1997). Briefly summarized, the hosts were aseptically vigorously brushed with alcohol sterilized #00 sable brushes in sterilized seawater baths. This was done on the stage of a good quality dissecting microscope in the wells of sterilized Pyrex® 9-hole spot plates. After transferring the hosts through 15 serial washes, the hosts were aseptically cut into 3 wedge-shaped pieces: one part was used for genetic extraction; one part was saved for morphological identification of the host; and remaining third was used for isolation of the symbionts. The latter piece was transferred aseptically into a fresh sterile well of a 9-hole spot plate containing 1 mM Na EDTA in seawater. The specimen was transferred every five minutes from one well to the next until the specimen had been completely decalcified (observed in a dissecting microscope). At this point the organic membrane bound specimen was torn apart with the aid of sterilized glass needles. With the aid of sterile capillary pipettes the symbionts were picked and transferred to test tubes containing one of the following media: F/2, M2 or Erdschreiber. After incubation in the light (18 h L/6 h D) for 7–10 days the isolates were streaked on media with agar. *Symbiodinium* spp. formed easily identifiable colonies on agar. Using these methods we isolated axenic clone cultures from: 1) *Amphisorus hemprichii* from Taba, Red Sea; 2) *A. saurensis*, from Bird Island, Lizard Island Group, GBR; 3) *A.* sp. from Zanpa Beach, Okinawa; 4–7) *Marginopora vertebralis* clones from Bird Island; Lizard Island (GBR), Palau, and Tahiti; and 8) an as yet unidentified *Sorites* sp or *Amphisorus* sp from Guam.

Preparation of antisera

As noted in previous experiments (Chai and Lee, 1999a,b, 2000) polyvalent antisera were prepared in rabbits by a commercial vendor (Pocano Rabbit Farms). The vendor was given centrifuged and washed frustrule fractions of three taxonomically diverse species of axenic cultured endosymbiotic diatoms, *Nanofrustulum shiloi*, *Nitzschia panduriformis*, *Amphora tenerimma*. The antisera used were from exsanguinated rabbits that had been given 2 booster shots of the frustrule preparation.

Control cultures

Control cultures were the same species of axenic cultured endosymbiotic diatoms, *Nanofrustulum shiloi*, *Nitzschia panduriformis*, *Amphora tenerimma* used to

prepare antisera in the rabbits. They were grown F/2 medium of Guillard (1975).

Test preparations

The indirect antibody method was used in these tests, algae were concentrated into 0.5 ml Ependorf centrifuge tubes and then 20 µl of rabbit antiserum to a symbiotic diatom was added. The mixture was vigorously mixed and then incubated at 37°C for one hour. After incubation the tubes were centrifuged, the supernatant was withdrawn and the cells were mixed with sterile filtered sea water. The cells were freed of unreacted serum by rinsing them 5 times with sterile seawater. Then 20 µl of FITC-tagged goat anti-rabbit-antibody was added. After thorough mixing the tubes were incubated at 37°C for an hour. Then they were rinsed 5 times to get rid of un-reacted antibody before mounting them on microscope slides for examination in an epifluorescence microscope.

3. Results and Discussion

While the polyvalent antisera against individual endosymbiotic diatoms reacted with all three of the control endosymbiotic diatom species tested there was no reaction with any of the endosymbiotic dinoflagellates (Table 1 and Fig. 1). One must conclude from this evidence that a different signaling molecule and receptor system is involved in the *Symbiodinium*-soritine symbiosis.

Table 1. Polyvalent antiserum (containing CSSA) against *Nanofrustulum shiloi*, *Nitzschia panduriformis* and *Amphora tennerima* tested against symbiotic dinoflagellates.

Dinoflagellates	*N. shiloi*	*N. panduriformis*	*A. tenerrima*
Control-*N. shiloi*	Positive	Positive	Positive
Control-*N. panduriformis*	Positive	Positive	Positive
Control- *A. tenerrima*	Positive	Positive	Positive
Zampa*	Negative	Negative	Negative
Amp hemp*	Negative	Negative	Negative
Guam*	Negative	Negative	Negative
Tahiti*	Negative	Negative	Negative
Liz Island*	Negative	Negative	Negative
Cass frond*	Negative	Negative	Negative
Bird Island*	Negative	Negative	Negative

*Strain nick-names: Zanpa=symbiont isolated from an *Amphisorus* sp on Zanpa Beach, Okinawa, Japan; Amp hemp=symbiont isolated from an *Amphisorus hemprichii* from the Red Sea at Eilat, Israel; Guam=symbiont isolated from an *Marginopora vertebralis* at Ritidian Beach, Guam; Tahiti=symbiont isolated from an M*arginopora vertebralis* on Tahiti; Liz Island=symbiont isolated from an *Marginopora vertebralis* seaward of the Marine Station on Lizard Island, Great Barrier Reef, Australia; Cass frond=symbiont isolated from an *Casseopeia frondosa* at Key West, Florida; Bird Island=symbiont isolated from an *Marginopora vertebralis* on Bird Island, Lizard Island Group, Great Barrier Reef, Australia.

These results may very well be in consonance with the results of a recently published molecular study that suggests

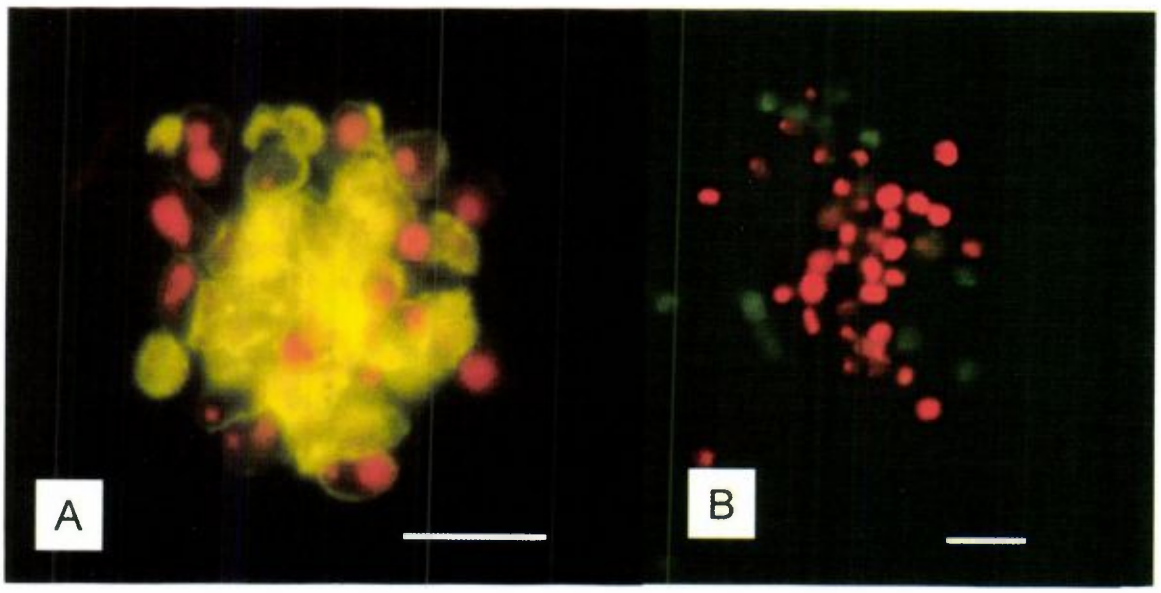

Figure 1. A. Positive reaction between polyvalent antiserum raised against *Nitzschia panduriformis*, a symbiotic diatom, tested against cultured *Nitzschia frustulum* symbiotica also a symbiotic diatom. The antiserum contains a common antibody against the surfaces of symbiotic diatoms. The antibody is tagged with FITC and viewed in an epifluorescence microscope. Cells are 10 µm.
B. Negative reaction between polyvalent antiserum raised against *Nitzschia panduriformis*, a symbiotic diatom, tested against cultured *Symbiodinium* sp., a symbiotic dinoflagellate from the larger foraminifer *Amphisorus hemprichii*. The antiserum contains a common antibody against diatom symbionts surfaces. The antibody is tagged with FITC and viewed in an epifluorescence microscope. Cells are 10 µm. The antibody fluoresces yellow-green; plastids of the symbionts are auto-fluorescing red. *See cover illustrations.*

that several *Symbiodinium* clades are restricted in their distribution to foraminifera belonging to the subfamily Soritinae (Garcia-Cuetos et al., 2005). Using both RFLP analysis and SSU rDNA sequencing they distinguished 22 phylotypes of Soritinae which were associated with 3 clades and 5 subclades of *Symbiodinium*. Fourteen of the 22 soritine phylotypes showed strict symbiont specificity. Only one of the soritine phylotypes was found to be the host for more than two groups of *Symbiodinium*. This may suggest, that because the symbionts and hosts have a finical relationship, there may not be some common antigen on the surfaces of the various clades of *Symbiodinium* analogous to the CSSA found on the surfaces endosymbiotic diatoms. Since the soritine hosts also are known to harbor secondary symbionts belonging to two widely divergent groups (Prymnesiophytes and Cyanobacteria) in addition to dinoflagellates (Lee et al., 1997), symbiont recognition, establishment and maintenance is likely to be multi-component process. Perhaps some common exported metabolite(s) is a component of the maintenance process. We look forward to answers to these intriguing questions.

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