Session 11

Animal-algal symbioses: molecular, physiological and genetic interactions, processes and adaptations

Session chairs:

Virginia Weis, weisv@science.oregonstate.edu

Simon Davy, simon.davy@vuw.ac.nz

John Pringle, jpringle@stanford.edu

Christian Voolstra, Christian.voostra@kaust.edu.sa

Tamar L. Goulet, tlgoulet@olemiss.edu

William Fitt, fitt@uga.edu

Todd C. Lajeunesse, tc13@psu.edu

Koty Sharp, kotysharp@gmail.com

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Symbiodinium kawagutii (clade F) coats the surface of *Acropora solitaryensis*, resulting in the formation of a sheet-like crust

I. Yuyama, T. Higuchi, T. Mezaki

Abstract *Symbiodinium kawagutii* (*Symbiodinium* sp, belonging to clade F) is one of the endosymbiotic algae isolated from reef-building corals. In order to investigate the characteristics of *S. kawagutii* as a symbiont, we tried to make corals associated with *S. kawagutii*, and compared them with other *Symbiodinium* associating corals. Aposymbiotic juvenile polyps (*Acropora solitaryensis*) were inoculated with monoclonal *Symbiodinium* cultures of CCMP2468 (*S. kawagutii*, clade F), CCMP 2466 (*S. goreaui*, clades C), and CCMP2556 (*S. trenchii*, clade D), and maintained in Petri dishes containing artificial seawater at 25°C for 5 months. We observed high densities of *S. goreaui* and *S. trenchii* inside each polyp. In contrast, *S. kawagutii* cells were not observed inside polyps. Instead, algal cell aggregations were found attached to the outside surface of polyps. Under the attachment sites of *S. kawagutii* aggregations, a sheet-like crust was observed on the coenesteum. Here we show that *S. kawagutii* does not form an endosymbiotic relationship with acroporid corals.

Keywords: endosymbiosis, *Symbiodinium*, Clade F, *Acropora solitaryensis*, juvenile polyp

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I. Yuyama
Center for Information Biology, National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-8540, Japan

T. Higuchi
Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, 277-8564, Japan

T. Mezaki
Biological Institute on Kuroshio, Kochi, Otsuki, Nishidomari, 788-0333, Japan

Corresponding author: I. Yuyama, ikuko_yy@hotmail.com

Introduction

There are various genetic types of the endosymbiotic dinoflagellate, *Symbiodinium* spp., so-called zooxanthellae (Taylor 1974; Rowan and Powers 1991). Although corals are commonly associated with clade C or clade D *Symbiodinium*, their symbiont type can change due to temperature, and host-growth stage and their habitat (Baker 2003; Pochon et al. 2004; Santos et al. 2004; Chen et al. 2005; Thornhill et al. 2006; Suwa et al. 2008; Abrego et al. 2009; Byler et al. 2013; Tonk et al. 2013; D'Angelo et al. 2015). Clade F *Symbiodinium* are occasionally detected with *Alveopora japonica* in temperate regions, suggesting that clade F is tolerant to low temperatures (Rodriguez-Lanetty et al. 2003; Lien et al. 2012). A *Symbiodinium* culture

(CCMP 2468, referred to as *Symbiodinium kawagutii*) of clade F5 was originally isolated from the Hawaiian stony coral, *Montipora verrucosa* (now *M. capitata* Trench and Blank 1987, Lin et al., 2015). However, clade F is not generally found in stony corals, but rather in foraminifera (Fay et al. 2009). *Symbiodinium* clade F remains poorly understood as a symbiont of corals and at the physiological level.

Some *Symbiodinium* cultures can be experimentally introduced as symbiont into aposymbiotic juvenile corals, and coral-monoclonal algae symbiotic systems can be established. Several studies have confirmed that juvenile corals can take up *Symbiodinium* clades A and D (*S. trentii*) within a few days after the start of inoculations (Yuyama et al. 2005, 2012, Yuyama and Higuchi 2014). Clade C (*S. goreaui*) can colonize corals, if they are co-cultured with corals for more than 2 months (Yuyama and Higuchi 2014). Acroporid corals have been used in such inoculation experiments because they can be artificially induced to metamorphose and remain in the aposymbiotic state as juvenile polyps (Iwao et al. 2002; Yuyama et al. 2005, 2011). *Acropora solitaryensis* used in this study is a dominant temperate species in Japan (Higuchi et al. 2015), and has recently expanded to higher latitude habitats (Yamano et al. 2011). If *A. solitaryensis* can acquire clade F *Symbiodinium*, it might develop increased stress tolerance to cold temperature. We report here the responses of *A. solitaryensis* to inoculation with clades *Symbiodinium* clades C (*S. goreaui*), D (*S. trenchii*), and F (*S. kawagutii*) cultures.

Materials and methods

The *Symbiodinium* strains CCMP2466 (*S. goreaui*, clade C), CCMP2556 (*S. trenchii*, clade D) and CCMP2468 (*S. kawagutii*, Clade F) (Trench and Blank 1987; LaJeunesse et al. 2014) were obtained from Bigelow Laboratory for Ocean Sciences (West Boothbay Harbor, ME, USA; https://ccmp.bigelow.org/) and cultured in f/2 medium (Wako Chemicals, Osaka, Japan) and antibiotics (kanamycin 20 µg ml^{-1} and ampicillin 50 µg ml^{-1}) at 24°C under a 12-h light (50 µE m^{-2} s^{-1}): 12-h dark cycle.

Collection of *A. solitaryensis* larvae was performed at The Biological Institute on Kuroshio (BIK) as previously described (Iwao et al. 2002). Seven days after spawning, larvae were exposed to 2 µM Hym 248, the Hydra derived GLW-amide neuropeptide, to induce metamorphosis in petri dishes containing filtered (pore size: 0.22 µm) seawater. Cells of each strain of *Symbiodinium* (approximately 2000 cells ml^{-1}) were introduced to juvenile polyps three days after metamorphosis. Each *Symbiodinium* culture was subsequently introduced to Petri dishes containing polyps every day. Two petri dishes containing a total of approximately 50 polyps were used for each treatment. A subset of the juvenile polyps were maintained in the aposymbiotic state for the duration of the experiment. All polyps were maintained at 25°C under a 12-h light (70 µE m^{-2} s^{-1}):12-h dark cycle for 5 months. To observe coral skeletons incubated with clade F *Symbiodinium* for 5 months, polyps were treated with 10% hypochlorous acid. Juvenile polyps were observed with a

stereomicroscope. Photographs of polyps were taken using a digital camera (Digital Slight DA-L1; Nikon, Tokyo, Japan).

Results

S. trenchii (clade D) rapidly populated *A. solitaryensis* juveniles and spread throughout the polyps within 2 weeks, whereas *S. goreaui* (clade C) colonization was very slow, showing little increase in polyps after 2 months. *S. kawagutii* (clade F) cells did not appear in the polyps. Instead algal cell aggregations were found attached to the outside surface of polyps (Fig. 1(i), (ii)). Budding and colony formation occurred in samples colonized by *S. goreaui* or *S. trenchii*, and their growth rate appeared higher than those inoculated with with *S. kawagutii* (Fig.1(iii), (iv)). Indeed, aposymbiotic polyps and polyps with *S. kawagutii* did not bud (Fig. 1 (i), (ii), (v)).

S. kawagutii cell aggregations were removed from the polyps with tweezers. After removal, we found that the surface of polyps were coated with a hard, thin, white crust. To more clearly observe the parts covered by *S. kawagutii* cell aggregation, 10% hypochlorous acid was used to remove the algae and soft tissues. As shown in Fig. 2, the coenosteum was covered by an additional sheet-like crust. These crusts were thin and easily broken. We observed normal coenosteum structure under the crusts.

Discussion

Although clade F *Symbiodinium kawagutii* were not incorporated as intracellular symbionts into corals, the co-culture of *S. kawagutii* with juvenile polyps presented us with an interesting result. Clade F is a rare *Symbiodinium* in reef-building corals, except in the temperate coral *Alveopora* (Pochon and Gates 2010; Lien et al. 2012; Rodriguez-Lanetty et al. 2003). In fact, other temperate corals including adult *A. solitaryensis* usually harbor *Symbiodinium* clade C (Lien et al. 2012). It is possible that *Symbiodinium* clade F cannot successfully colonize *A. solitaryensis*. Our results also show that clade F (*S. kawagutii*) -attached corals did not form any budding polyps and their growth rate was lower than that of polyps associating with *S. goreaui* and *S. trenchii* (. 1), suggesting that attachment of *S. kawagutii* and co-culture with *S. kawagutii* did not promote coral growth. Sheet–like crusts were observed on coenostea under the attachment of the *S. kawagutii* aggregations (Fig. 2). Normally, the body walls of *A. solitaryensis* consist of a reticulated and highly porous coenosteum. Two hypotheses we are considering to explain the formation of these sheet-like crusts are 1) the corals make these crusts to prevent the direct attachment of *S. kawagutii* cells, 2) the crusts are made from a composite of *S. kawagutii* cells and other microbes.

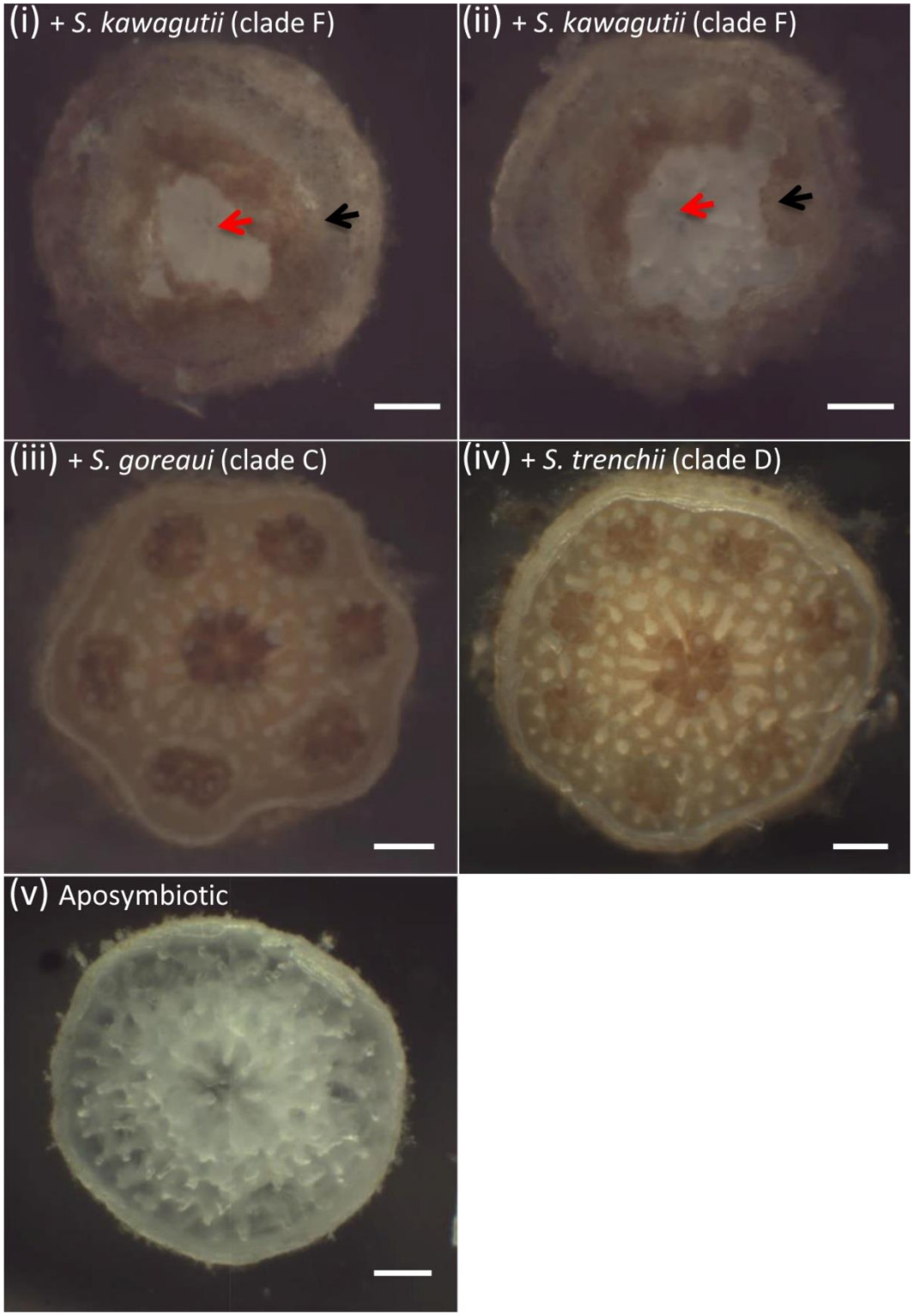

Fig. 1 Juvenile *Acropora solitaryensis* polyps inoculated with *Symbiodinium kawagutii* (i)(ii), *S. goreaui*, (iii) or *S. trenchii* (iv) monoclonal cells. Aposymbiotic polyps maintained during the same period are shown in (v). Photographs of polyps were taken 150 days after inoculation. Black arrows indicate clade F *Symbiodinium* covering the coral polyps. Red arrows indicate polyp mouths. Scale bars = 0.25 mm.

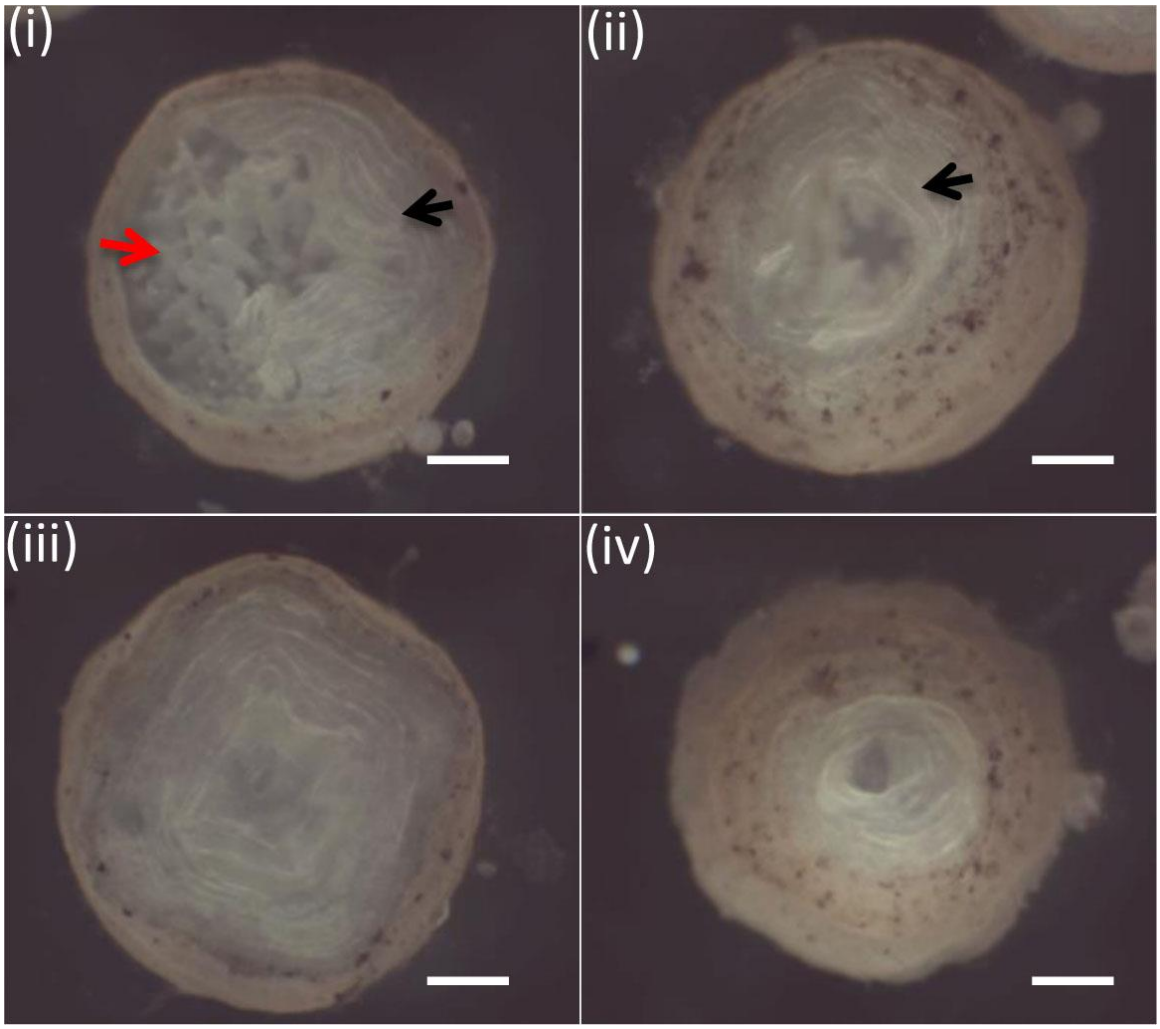

Fig. 2 Skeletons of juvenile *Acropora solitaryensis* inoculated with *S. kawagutii*. Soft tissues were removed with 10% hypochlorous acid. Black arrows indicate sheet-like crusts (i,ii). Red arrow shows the coenesteum after the sheet-like crusts were broken. The sheet-like crusts covered the mouth of the polyps (iii,iv). Scale bars = 0.25 mm.

Coral calcification processes generally take place between calicoblastic epithelium layers and the skeleton (Venn et al. 2011; Vandermeulen et al. 1975; Allemand et al. 2004). As these sheet-like crusts broke easily and normal coenosteua existed under them, it seemed that the sheet-like crusts formed on the surface of the soft tissue of the coral body walls. It remains unclear whether or not these sheet-like crusts are also formed through the normal calcification process via calicoblastic layers. Further histological analysis would be helpful to confirm the localization of calicoblastic cells near the sheet-like crusts and to clarify the origin of the crusts. Another possibility for the origin of the crusts is a composite of *S. kawagutii* cells and microbes, because free-living *Symbiodinium* in culture have been shown to form calcifying microbial–algal communities that deposit aragonitic spherulites called symbiolites (Frommlet et al., 2015). The mechanisms that produce the symbiolites is unclear, but this ability suggests that the sheet-like crusts could be produced by something other than corals.

Although *Symbiodinium* clade F (*S. kawagutii*) used in this study may not correspond with the symbiont clade F inhabiting the reef-building coral, we showed that *A. solitaryensis* did not form an endosymbiotic relationship with *S. kawagutii*. After the 5 month incubation, free-living *S. kawagutii* cells coated the coral bodies, resulting in the formation of a sheet–like crust on the coenesteum.

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