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Original article

Juvenile *Acropora tenuis* attract *Symbiodinium* by using GlcNAc-binding lectin

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Introduction

Most reef-building corals acquire symbiotic dinoflagellates, *Symbiodinium* spp., from surrounding environments and establish symbiosis with *Symbiodinium* [1-3]. Although, *Symbiodinium* are present at low densities under natural environmental conditions, corals acquire *Symbiodinium* effectively [4]. This therefore raises the possibility that corals acquire *Symbiodinium* by utilizing chemotactic compounds.

Recently, Yamashita et al. reported that juvenile *Acropora tenuis*, appears to attract *Symbiodinium* during the initial stage of symbiosis [5]. Furthermore, it has been reported that *N*-acetyl-D-glucosamine (GlcNAc) binding lectin in *A. tenuis*, ActL, attract *Symbiodinium* NBRC102920 strain which is acquired by juvenile *A. tenuis* polyps [6]. Interestingly, Kuniya et al. showed that juvenile *A. tenuis* polyps acquire only few *Symbiodinium* strains among tested [7], suggesting that *A. tenuis* contains chemotactic compounds that can attract specific *Symbiodinium* strain. In the present study, we examined the chemotactic activity of ActL to six *Symbiodinium* culture strains and examined whether juvenile *A. tenuis* polyps discharge ActL to consider ActL could be a factor of symbionts selection.

Materials and methods

A. tenuis specimens were collected from Sesoko Island. Collected *A. tenuis* were maintained in an aquarium for several days and then frozen at -70°C in a freezer until use. *Symbiodinium tridacnidorum* strain NBRC102920 (clade A), GTP-A6-Sy (clade A), CCMP1633 (clade B), CCMP2556 (clade D), CCMP421 (clade E) and CS-156 (clade F) were used for the chemotactic assay.

Purification of ActL
Purification of ActL was performed according to Takeuchi et al. [6]. ActL was purified by GlcNAc affinity chromatography, and then dialyzed against IMK medium to use for the chemotactic assay.

Chemotactic activity assay method
We added 100 µl of a *Symbiodinium* strain at 1.0×10^6 cells/ml to a 1.5-ml PROKEEP tube (Fukae Kasei Co., Ltd, Hyogo, Japan) and centrifuged the tube at 860 ×*g* for 5 min at 25°C. After removing the supernatant, 100 µl of IMK medium was added to the pellet. After 24 h, Capillary Calibrated Pipettes (Drummond Scientific Company, Broomall, PA, USA) containing 2-µl samples of ActL or IMK medium were inserted into the tubes containing *Symbiodinium*. After 60 min, each capillary was removed from the tubes, and the sample in the capillary was blown by mouth into a hemocytometer and the *Symbiodinium* cells were counted. The number of attracted cells was quantified by subtracting the number of *Symbiodinium* cells in a capillary containing IMK medium from that containing ActL. The chemotactic activity was defined according to previous paper [6]. When the number of attracted cells was 40 cells, the chemotactic activity was defined as 1 unit.

Concentration of released ActL by A. tenuis
The concentrations of ActL, which was released from juvenile polyps, were quantified by the enzyme linked immunosorbent assay (ELISA). Ten metamorphosed juvenile polyps were placed in a well of 8-well chambered coverglass (Nunc, Rochester, NY, USA), incubated in 500 µl of artificial sea water (ASW; Wako Pure Chemical Industry, Osaka, Japan) at 25°C for 24 h, and then the chamber was replenished with 500 µl of ASW. After replenished, the chambered coverglass with juvenile polyps was incubated for 24 h, then ASW were collected and used for ELISA using anti-ActL antibody to quantify the concentration of ActL.

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Results

The chemotactic activity of ActL to NBRC1029020
ActL attracted a greater number of NBRC102920 cells than did IMK medium, and the activity was significantly inhibited by GlcNAc at 10 mM and anti-ActL antibody at 5 µg/ml (Fig. 1).

The difference of chemotactic activity to ActL by Symbiodinium strains
The chemotactic activity was the greatest for NBRC102920 (3.0±0.1 U) and CCMP2556 (2.5±0.5 U), followed by CCMP1633 (0.9±0.5 U), GTP-A6-Sy (0.1±0.1 U), CCMP421 (-0.1±0.03 U) and CS-156 (-0.1±0.2 U).

Concentration of released ActL from juvenile polyps
We examined whether ActL was released from juvenile polyps by dot blotting using the anti-ActL antibody. ASW in which juvenile polyps were reared react to anti-ActL antibody, while ASW without juvenile polyps did not. Since anti-ActL antibody preincubated with ActL abolish the positive reaction, juvenile polyps release ActL outside a body. Quantified by ELISA, the concentration of ActL in ASW rearing polyps was 3.4 ng/ml/polyps. At this concentration, the ActL attracted NBRC102920 (0.9±0.03 U). The number of *Symbiodinium* in the capillary with ActL was significantly higher than that with IMK medium.

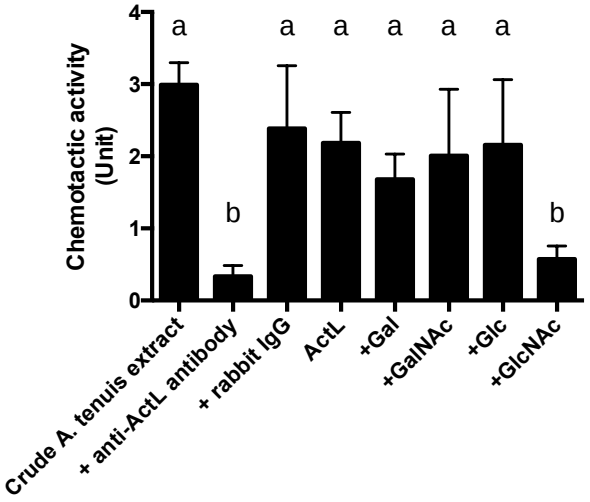

Fig. 1. The chemotactic activity of each sample. Anti-ActL antibody or rabbit IgG (at a final concentration of 5 µM) were added to crude *A. tenuis* extract prior to conducting the chemotactic assay. To perform inhibition assay, sugars (at a final concentration of 10 mM) were added to ActL prior to conducting the chemotactic assay. *Symbiodinium* strain NBRC102920 was used for the assay. Values are the mean ± SD (n = 3). Different letters indicate a significant difference between the chemotactic activities (P < 0.05, Tukey's multiple comparisons test).

Discussion

We found that strains NBRC102920 and CCMP2556 were well attracted by ActL. Interestingly, these strains are known as well acquired by juvenile *A. tenuis* polyps [7]. Since there seems to be correlation between attraction and acquisition [7], the chemoattraction seems to select acquiring *Symbiodinium*. Moreover, a juvenile polyp released ActL at 3.4 ng/ml, which is enough to attract *Symbiodinium*. Hence, juvenile polyps might attract and acquire specific *Symbiodinium* by using ActL. These results suggested that ActL is a factor of *Symbiodinium* selection and plays an important role in *Symbiodinium* acquisition.

The mechanism of chemoattraction is still unclear, but treatment of *Symbiodinium* with glycosidase reduced the number of *Symbiodinium* cells acquired by sea anemone and corals [7-9]. Therefore, a sugar binding protein ActL should bind to surface sugar chain of *Symbiodinium* to attract. Further studies are needed to understand the mechanism of coral chemoattraction.

In this study, we found ActL attract specific *Symbiodinium* strains which can be acquired by *A. tenuis*. Approximately 75% of spawning corals acquire *Symbiodinium* from the surrounding environments (horizontal transmission) [3], and mainly establish symbiosis with definite *Symbiodinium* [10]. It seems that all the corals species select *Symbiodinium* by some kinds of chemical substances. Probably, chemoattraction may be the key to select *Symbiodinium*, and important for corals to establish symbiosis with *Symbiodinium*.

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