Motility and cell division patterns among several strains of *Symbiodinium*

Hiroshi YAMASHITA¹,* and Kazuhiko KOIKE²

¹Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Japan Fisheries Research and Education Agency, 148 Fukai-Ohta, Ishigaki, Okinawa 907–0451, Japan
²Graduate School of Biosphere Science, Hiroshima University, 1–4–4 Kagamiyama, Hiroshima 739–8528, Japan

* Corresponding author: H. Yamashita
E-mail: hyamashita@fra.affrc.go.jp

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**Abstract** The dinoflagellate genus *Symbiodinium* consists of well-known coral symbionts. The genus is divided into nine phylogenetically distinct groups (clades A–I), with each consisting of numerous small groups (types or subclades). Some *Symbiodinium* can be isolated from host animals and maintained as culture strains under laboratory conditions, but there is currently very little biological information on cultured *Symbiodinium*. In the present study, we observed motility and cell division patterns in eight *Symbiodinium* culture strains for clades A–F. Although cell division patterns were congruent among all of the culture strains, their motility patterns differed. Peak cell division was observed at dawn in all of the cultures. The ratio of motile phase cells declined at night, but in clade A type A2 relative and clade E *Symbiodinium* culture strains, which were isolated from the environment, >10% of the cells actively swam at night. While we only investigated eight cultures, we observed similarities and differences in their characteristics. Although several *Symbiodinium* are difficult to cultivate, future studies on cultured cells would give better insights into the biology of *Symbiodinium* spp.

**Keywords** Zooxanthellae, *Symbiodinium*, dinoflagellate, culture strains, microscopy

**Introduction**

The dinoflagellate genus *Symbiodinium* comprises well-known symbionts of marine invertebrates, including corals. Loss of *Symbiodinium* from corals, usually referred to as coral bleaching, often leads to mass mortality in corals (e.g., Hoegh-Guldberg 1999; Douglas 2003), which indicates that the host animals depend on the *Symbiodinium*. However, some *Symbiodinium* can survive without host animals; that is, certain *Symbiodinium* cells can be isolated from host animals and maintained under laboratory conditions as culture strains (Schoenberg and Trench 1980a). Cultured *Symbiodinium* exhibit daily morphological changes between a flagellated (motile) stage during the day to a non-flagellated spherical (coccoid) stage at night (Freudenthal 1962). *Symbiodinium* cells in the motile stage have thin thecal plates (Loeblich and Sherley 1979; Trench and Blank 1987), and thus, some *Symbiodinium* species have been formally described with thecal plate tabulations for species criterion (Hansen and Daugbjerg 2009; Jeong et al. 2014; Lee et al. 2015). However, for the *Symbiodinium* genus, the differences in thecal tabulations between genetically distant species are small (Jeong et al. 2014); therefore, molecular grouping
has been commonly used. Several molecular markers, such as nuclear ribosomal RNA genes (Rowan and Powers 1991; Wilcox 1998; LaJeunesse 2001), plastid-encoded genes (Santos et al. 2002; Takishita et al. 2003), and mitochondrial genes (Takabayashi et al. 2004), have been used for either phylogenetic analysis or grouping of *Symbiodinium*. As a result, nine groups (clades A–I) are currently recognized (Pochon and Gates 2010), and each clade consists of numerous types (LaJeunesse 2005). The number of formally described *Symbiodinium* species is, unfortunately, still far below that of the genetically classified groups, but it is widely accepted that the *Symbiodinium* genus is composed of divergent members.

As coral bleaching has become a global issue, research has generally concentrated on the symbiotic relationship between corals and *Symbiodinium*, and/or the ecophysiology of the holobiont (symbiotic community) itself. As a result, coral bleaching mechanisms and the underlying coral/algal behaviors have been gradually elucidated (e.g., Gates et al. 1992; Fitt et al. 2001; Fujise et al. 2014). However, information on the basic biology of the *Symbiodinium* genus remains limited. Several *Symbiodinium* species descriptions have recently been published (Hansen and Daugbjerg 2009; LaJeunesse et al. 2012; LaJeunesse et al. 2014; Jeong et al. 2014; Lee et al. 2015; LaJeunesse et al. 2015; Parkinson et al. 2015). The biological characteristics of *Symbiodinium* might be among the potential criteria for species discrimination, and should be taken into account. In the present study, we compared behavioral characteristics, such as motility and cell division patterns, among *Symbiodinium* culture strains belonging to several genetic clades.

**Materials and Methods**

*Symbiodinium* culture strains

Eight *Symbiodinium* culture strains belonging to clades A–F were used; the details of which are listed in Table 1. Briefly, clade A type A1 (AJIS2-C2), type A2 relative (GTP-A6-Sy), type A3 (CS-161), clade B (CCMP1633), clade C type C1 (CCMP2466), clade D type D1-4 (formerly D1a; CCMP2556), clade E (MJa-B6-Sy), and clade F (CS-156) strains were used. The CS-161 and CS-156 cultures were purchased from the Commonwealth Scientific & Industrial Research Organization (ACT, Australia), and CCMP1633, CCMP2466, and CCMP2556 were purchased from the Provasoli–Guillard National Center for Culture of Marine Algae and Microbiota (ME, USA). The other cultures—AJIS2-C2, GTP-A6-Sy, and MJa-B6-Sy—were originally isolated by Yamashita and Koike (2013). All cultures were maintained in a 27°C incubator under a light regime of 80–120 µmol photon m⁻² sec⁻¹, provided by cool white fluorescent lamps (5000 K, 12-h light: dark period; 07:00 light on, 19:00 light off) in IMK medium (Sanko Jyunyaku, Tokyo, Japan).

**Light microscopy**

Light micrographs of each culture strain were taken under a differential interference contrast (DIC) microscope (BX-51 or BX-50, Olympus, Tokyo, Japan), with an ultra-sensitive CCD camera (CoolSNAP ES, Roper Scientific, AZ, USA; or DP-73, Olympus). To record both motile and coccoid phase cells, we observed all of the culture strains during the day and at night. Some of the *Symbiodinium* cultures have an eyespot situated around the sulcus (longitudinal groove), which is only present during the motile phase (Hansen and Daugbjerg 2009; Yamashita et al. 2009; Jeong et al. 2014). The *Symbiodinium* eyespots can be recognized as a bright light spot when the DIC analyzer is removed from the microscope (Yamashita et al. 2009), and were also observed in this study.

**Observations of motility and cell division patterns**

Prior to observations, the *Symbiodinium* culture strains were incubated for 2 weeks in the above mentioned incubator. The strains were then diluted with IMK medium and transferred into a 24-well culture plate (Costar 3526, Corning Incorporated, NY, USA) at a concentration of 5000 cells mL⁻¹ (three wells for one culture; triplicate), and the plate was incubated for an additional 3 days to acclimate the cells. Live cell behavior was observed at 3-h intervals for 27 h under an inverted light microscope (IX-70, Olympus). Over 200 cells from randomly selected areas in each well were observed and the number of motile, coccoid, and dividing phase cells were counted. The ratios of motile and dividing cells in each observation period were calculated as [number of motile cells / total
observed cell number × 100], and [number of dividing cells / total observed cell number × 100], respectively. The mean and standard deviation (SD) for each observation period were calculated from the results of three wells per Symbiodinium culture strain (triplicate measurements). The results (mean and SD) were calculated from technical replicates because all of the Symbiodinium culture strains used in the present study were clonal cultures.

### Results and Discussion

All of the cultures exhibited daily morphological changes. Figure 1 shows the light micrographs of each Symbiodinium culture strain. The cells swam actively in the daytime. Bright spots near the sulcus were observed in the motile cells during the daytime when the DIC analyzer was removed from the microscope, but these spots were not observed in the coccoid stage cells at night. This indicates that all of the cultures tested had eyespots, which consisted of layered crystalline deposits, during only the motile stage (Yamashita et al. 2009). Most of the cells turned into spherical non-flagellated coccoid phase cells at night, although a few cells from clade A type A3, clade B, clade C, and clade F Symbiodinium strains remained in the motile phase at night. The motile cell rates in these culture strains at night were very small, from 0.2 ± 0.3% in clade C to 3.6 ± 1.0% in clade F, however, in the GTP-A6-Sy (clade A, type A2 relative) and MJa-B6-Sy (clade E) cultures, at least 12.7 ± 1.4% (type A2 relative) and 19.4 ± 1.7% (clade E) of the cells actively swam at night (Figure 2). Nocturnal motility has been reported in clade E Symbiodinium, i.e., Symbiodinium voratum Jeong, Lee, Kang, LaJeunesse (2014) (Jeong et al. 2014), which agrees with our current finding. Additionally, our observations revealed that type A2 relative Symbiodinium, i.e., Symbiodinium natans Gert Hansen and Daugbjerg (2009) also exhibit nocturnal motility in culture conditions. S. voratum and S. natans have mainly been isolated from non-host habitats, such as the water column, tide pools, and macroalgal surfaces (Hansen and Daugbjerg 2009; Yamashita and Koike 2013), and, thus, are likely a free-living specialized group. If so, nocturnal motility might be a common characteristic of free-living Symbiodinium species. However, AJIS2-C2 (clade A type A1, i.e., Symbiodinium microadriaticum Freudenthal (1962), emend. by Trench and Blank (1987), emend. by Lee, Jeong, Kang, LaJeunesse (2015)) and CCMP2556 (clade D type D1-4, i.e., Symbiodinium trenchii LaJeunesse (2014)) completely changed into the coccoid form at night. These Symbiodinium species are acquired well by Acropora coral larvae under laboratory conditions, and are commonly found in naturally settled Acropora recruits in the field (Yamashita et al. 2014). Cultured coccoid stage cells are similar to the symbiotic cell morphology within host animals, although the pellicle layer is thinner in the host tissue (Schoenberg and Trench 1980b). Considering this fact, the corals may prefer S. microadriaticum (type A1) and S. trenchii (type D1-4), which only change form into coccoid cells, rather than the nocturnal S. natans (type A2 relative) and S. voratum (clade E) as their initial partner.

The motility patterns differed among clades, and even

| Strain     | Clade/type | Species name                     | Isolation source |
|------------|------------|----------------------------------|------------------|
| AJIS2-C2   | A1         | Symbiodinium microadriaticum     | Acropora sp.     |
| GTP-A6-Sy  | A2 relative| Symbiodinium natans              | Tide pool        |
| CS-161     | A3         | Symbiodinium tridacnidorum       | Tridacna derasa  |
| CCMP1633   | B          | Symbiodinium sp.                 | Aiptasia pulchella|
| CCMP2466   | C1         | Symbiodinium goreau              | Discosoma sanctithomae|
| CCMP2556   | D1-4       | Symbiodinium trenchii            | Montastraea faveolata|
| MJa-B6-Sy  | E          | Symbiodinium voratum             | Macroalgal surface|
| CS-156     | F          | Symbiodinium kawagutii           | Montipora verrucosa|
among types within the same clade. However, cell division patterns were similar among the strains tested. Although there were differences in the percentages among strains, the ratio of dividing cells gradually increased during the night, and peaked toward morning (Figure 2). This pattern was also found in the nocturnal type A2 relative and clade E \textit{Symbiodinium} and, thus, the nocturnal motility in these \textit{Symbiodinium} strains is not attributable to a disturbed daily rhythmicity. In the present study, the maximum rate of dividing cells was 66.3±2.4% in clade E, and the minimum rate was 16.4±2.3% in clade C; furthermore, the maximum and minimum rates of motile cells were also observed in clade E (84.6±5.5%) and clade C (16.6 ±1.9%), respectively. Fitt and Trench (1983) suggested the possibility that motility levels may reflect growth rate, and, thus, our results for these two strains are consistent with this theory. In our observations, however, the maximum rates of dividing cells of clade A type A3, clade D type D1-4, and clade F \textit{Symbiodinium} strains were almost equal (41.2±4.7%, 40.8±1.3%, and 43.9±4.2%, respectively), whereas the motile rates varied among these strains (60.5±4.7%, 66.7±3.0%, and 77.3±4.7%, respectively). Additionally, the maximum dividing/motile cell rates differed among type A1, type A2 relative, and type A3 \textit{Symbiodinium} strains, despite these strains belonging to the same clade A. The maximum motility rate often depends on the culture environment, e.g., medium and/or irradiance (Fitt et al. 1981; Fitt and Trench 1983; Yacobovitch et al. 2004). Moreover, either tetrad cells or cell colonies are often found in the \textit{Symbiodinium} culture.

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**Fig. 1** Light micrographs of cultured \textit{Symbiodinium} cells during the day and at night. Strain names and clade/type are shown under the three pictures. The left column shows motile stage cells during the day, the middle column shows eyespots (arrows) in motile stage cells, and the right column shows coccoid stage cells at night.
vessel (Freudenthal 1962), suggesting that some \textit{Symbiodinium} cells do not swim in the morning even if they divided the previous night. Considering these facts, the rates of motile and dividing cells often change under different conditions, although motility and cell division are basically correlated.

In the present study, we observed eight culture strains belonging to six genetically distinct clades. Our results were obtained under specific culture conditions, however, we observed both similarities and differences in characteristics among the cultures. Although the \textit{Symbiodinium} genus is composed of divergent members, some of the groups, particularly the symbionts within host animals, cannot be isolated or established as culture strains (Santos et al. 2001; LaJeunesse 2002; Jeong et al. 2014). Under the circumstances, genetic-based alphanumeric methods for \textit{Symbiodinium} grouping have been widely adopted. However, there is little consensus among them and, therefore, nomenclatural clarity and taxonomic definitions are needed (LaJeunesse et al. 2012). However, the difficulties in establishing \textit{Symbiodinium} genus cultures differ greatly among the clades and even among types within the same clade. Unfortunately, no culture strains are available for most of the \textit{Symbiodinium} genetic groups. However, important biological characteristics can be obtained from culture observations and experiments if cultured strains are maintained in the laboratory, and such information might be potential criteria for species discrimination. Therefore, efforts to isolate and establish the culture strains are still necessary to clarify the diversity and biology of the \textit{Symbiodinium} genus.

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Fig. 2 Trends in motile and dividing cell rates in the \textit{Symbiodinium} culture strains.
The strain names and clade/type are shown in the upper part of each graph. The white and black bars on the observation times indicate the light on (white) and light off (black) in the incubator. The line graphs show the percentage of motile stage cells, and the bar graphs show dividing cells. The bars on each data point indicate the standard deviations of triplicate measurements.
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