A Mating Group Newly Found in the Subtropical Form of
Dictyostelium purpureum Olive

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Abstract Nineteen strains morphologically grouped into the subtropical form of Dictyostelium purpureum were paired with each other for mating test or macrocyst formation test. Pairings of the 7 strains resulted in macrocyst formation. These heterothallic strains belonged to a single mating group, in which 4 mating types were recognized and a strain of each mating type was compatible with strains of all other mating types. The macrocysts were easily distinguishable from those of other dictyostelids by the outermost thick walls consisting of many vacuolated cells.

Key words: dictyostelids, Dictyostelium purpureum, heterothallic, macrocysts, subtropical form.

Dictyostelium purpureum Olive was originally described on the basis of the isolates from the United States of America (Olive, 1901). This species is one of cosmopolitan dictyostelids and globally ubiquitous at lower latitudes, but it has not been discovered at higher latitudes in Europe and North America except for in larch and pine forests of central Sweden (Swanson et al., 1999). Also in Japan, according to Cavender & Kawabe (1998), the average importance value of D. purpureum was 22 in the warm temperate zone, 18 in the cool temperate zone and 0 in the boreal zone, though D. purpureum was found at both subalpine forests of Mt. O-Akan in Hokkaido (Kanda and Sato, 1982) and Mt. Senmai-dake and Mt. Fuji in Honshu (Kawabe, 1980, 1993).

Japanese isolates of D. purpureum were grouped into two forms, the temperate form and the subtropical form, based on a combination of four morphological characters of the sorocarps; namely, sorus color, sorophore length, supporter size and spore shape (Hagiwara, 1992). In the temperate form, one mating group was recognized and it was confirmed that this mating group was distributed in Japan and Korea (Hagiwara et al., 2004). On the other hand, the subtropical form was often isolated from Okinawa in the southernmost part of Japan (Hagiwara, 1992), but its mating system was not known.

In the course of our investigation in order to establish a biological species concept of dictyostelids, the macrocyst formation representing the sexual stage was discovered in the subtropical form of D. purpureum. The mating system was heterothallic and the macrocyst was unique in morphology among dictyostelids. We report here the mating group newly found in this study.

Materials and Methods

Nineteen strains of Dictyostelium purpureum were used in this study (Table 1). They consisted of 9 Japanese strains, 2 Korean strains, 3 Chinese strains and 5 Pakistani strains. They were macroscopically identified with the subtropical form of

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**D. purpureum** on the basis of the morphological categories of Hagiwara et al. (2004). In sexuality, all strains were neither homothallic nor belonging to the mating group in the temperate form of *D. purpureum* as a result of preliminary mating test.

Procedures of cultivation and observation followed Hagiwara et al. (2004). To test the mating competence, spores of each pair of strains were inoculated into small colonies of *Escherichia coli* on 0.1% lactose/0.1% proteose peptone agar plates. For underwater cultures, 5 ml of sterile Bonner’s salt solution was added to each plate after the spores had germinated. Cultures were incubated at 25°C in the dark and observed after 3 weeks incubation.

Macrocysts were mounted in distilled water and measured. Fifty macrocysts per pair of strains were used for calculating the mean diameter.

**Results and Discussion**

JKS 275 was used for a mating test strain because it was one of the most typical strains in the subtropical form of *Dictyostelium purpureum* (Hagiwara et al., 2004). As a result of pairing...
with all 19 strains examined, JKS 275 was compatible with JKS 50, JKS 274-2, CUH 96, CUH 167 and M 97. Among these 5 strains, JKS 274-2 was selected as a second test strain. As a result of pairing with all strains, JKS 274-2 was compatible with JKS 275, KMM 1, CUH 96, CUH 167 and M 97. These results suggested that 7 strains, namely, JKS 50, JKS 274-2, JKS 275, KMM 1, CUH 96, CUH 167 and M 97, were belonging to a single mating group but the other 12 strains were clearly not belonging to this group in the mating system (Table 2). These 12 strains were excluded from the following mating test.

Table 3. Macro cyst formation from pairings of four different mating types in the subtropical form of Dictyostelium purpureum.

<table>
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<th>S1 JKS 274-2</th>
<th>S2 JKS 275</th>
<th>S3 CUH 96</th>
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<td>S2 JKS 275</td>
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<td>S3 CUH 96</td>
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<td>S4 M 97</td>
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Fig. 1. Macrocysts of the mating group newly found in the subtropical form of Dictyostelium purpureum. A. Mass of macrocysts. ×115. B. Macro cysts different in morphology and developmental stages. ×460. C–F. Macrocysts. Note their outermost walls consisted by many vacuolated cells. Figs. D and F show the surfaces of the macrocysts in Figs. C and E, respectively. ×460. Fig. A, a pair of strains JKS 275 and M 97; Fig. B, a pair of strains JKS 274-2 and JKS 275; Figs. C and D, a pair of strains JKS 274-2 and CUH 96; E and F, a pair of strains KMM 1 and CUH 96.
As three of the above 7 strains, CUH 96, CUH 167 and M 97, were compatible with both of JKS 275 and JKS 274-2, CUH 96 was tentatively selected as a third test strain and paired with each of the 7 strains. CUH 96 was compatible with 5 strains including M 97, but not with CUH 96 and CUH 167 (Table 2). Lastly, M 97 was used for a fourth test strain and, as expected, M 97 was compatible with the other strains.

The results of the above mating tests are shown in Table 2. These suggested that there were four mating types belonging to a single mating system, summarized in Table 3. Such a mating system is similar to that of *D. giganteum* Singh (Erdos et al., 1975). We supposed that this mating system was heterothallic and multipolar like that of the temperate form of *D. purpureum* found by Hagiwara et al. (2004).

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**Fig. 2.** A mating group newly found in the subtropical form of *Dictyostelium purpureum*. A. Upright part of a prostrate sorophore. Note a well-developed supporter indicated with an arrow. ×45. B. Spores. ×1150. C. Abnormal spores. ×1150. D. E. Sorophore tips. ×460. F. Sorophore base expanding conically or with a small disk. ×460. G and H. Higher magnifications of the well-developed supporter indicated with an arrow in Fig. A. Fig. G, ×115; Fig. H, ×460. Figs. A, G and H, strain CUH 167; Fig. B, strain JKS 50; Fig. C, strain KMM 1; Figs. D and F, strain JKS 274-2; Fig. E, strain JKS 275.
Among the 12 strains excluded from the mating group newly found in this study, there may be some non-sexual strains of the tropical form or other mating group(s). This possibility remains to be investigated.

Macrocysts of the mating group newly found in the subtropical form of *D. purpureum* had a characteristic structure. Namely, their outermost walls consisted of many vacuolated cells (Fig. 1). Such a structure is not known in other dictyostelids except for the temperate form of *D. purpureum*, in which some vacuolated cells were sometimes found in the outermost walls of macrocysts (Hagiwara *et al.*, 2004).

Seven strains comprising the mating group newly found in this study fitted the original description of the subtropical form of *D. purpureum* (Hagiwara, 1992) (Fig. 2). The macrocysts were usually globose, mostly 19–57 μm in diam (Min.: 12 μm. Max.: 68 μm. Range of the mean diameter: 29–44 μm). In these dimension values, the macrocysts of the subtropical form were not distinguishable from those of the temperate form.

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**References**


