

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

Hiromitsu Hagiwara<sup>1</sup>, Shin-ichi Kawakami<sup>2\*</sup>, Ji-Young Hwang<sup>3\*\*</sup> and Yu Li<sup>4</sup>

<sup>1</sup>Department of Botany, National Science Museum, Amakubo 4–1–1, Tsukuba, Ibaraki, 305–0005 Japan  
E-mail: h-hagiwa@kahaku.go.jp

<sup>2</sup>Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305–8572 Japan

<sup>3</sup>Department of Biological Sciences, College of Natural Sciences, Kyungnam University, Masan, 631–701 Korea

<sup>4</sup>Jilin Agricultural University, Changchun, Jilin, 130118 P. R. China

**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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\*Present address: National Institute of Agrobiological Sciences, 2–1–2 Kannondai, Tsukuba, Ibaraki, 305–8602 Japan.

\*\*Present address: Department of Crop Life Safety, National Institute of Agricultural Science and Technology, Suwon, 441–701 Korea.

Table 1. Strains of the subtropical form of *Dictyostelium purpureum* examined for mating test.

Strain	Date of isolation
Japanese strains	
IsB 11	Okinawa Pref., Ishigaki Isl., 1994, by S. Kawakami
JKS 50	Wakayama Pref., Wakayama-shi, 1998, by HH*
JKS 56	Wakayama Pref., Wakayama-shi, 1998, by HH
JKS 80	Hyogo Pref., Awaji Isl., 1998, by HH
JKS 274-2	Wakayama Pref., Wakayama-shi, 1999, by HH
JKS 275	Wakayama Pref., Wakayama-shi, 1999, by HH
OH 7	Okinawa Pref., Hateruma Isl., 1986, by O. Yamazaki
TI 110	Tokyo Pref., Hachijo Isl., 2002, by HH
TI 115	Tokyo Pref., Hachijo Isl., 2002, by HH
Korean strains	
KMM 1	Kyungnam Pref., Masan, Mt. Muhak, 2000, by J. Hwang
KMM 8	Kyungnam Pref., Masan, Mt. Muhak, 2000, by J. Hwang
Chinese strains	
CUH 96	Yunnan Prov., Qujing Pref., Huize Co., 1998, by HH
CUH 150	Yunnan Prov., Nujang Pref., Lushui Co., 1998, by HH
CUH 167	Yunnan Prov., Nujang Pref., Gongshan Co., 1998, by HH
Pakistani strains	
B 17	Punjab, Islamabad, 1992, by HH
C 143	Northwest Frontier Province, Peshawar, 1992, by HH
M 97	Punjab, Rawalpindi, 1991, by HH
Q 67	Sind, Hyderabad, 1992, by HH
V 32	Northwest Frontier Province, Mansehra, 1992, by HH

\* H. Hagiwara.

*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.

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

## Results and Discussion

JKS 275 was used for a mating test strain be-

Table 2. Macrocyt formation from pairings of 19 strains of the subtropical form of *Dictyostelium purpureum*.

	JKS 275	JKS 274-2	CUH 96	M 97
JKS 50	+	-	+	+
JKS 274-2	+	-	+	+
JKS 275	-	+	+	+
KMM 1	-	+	+	+
CUH 96	+	+	-	+
CUH 167	+	+	-	+
M 97	+	+	+	-
IsB 11	-	-		
JKS 56	-	-		
JKS 80	-	-		
OH 7	-	-		
TI 110	-	-		
TI 115	-	-		
KMM 8	-	-		
CUH 150	-	-		
B 17	-	-		
C 143	-	-		
Q 67	-	-		
V 32	-	-		

cause 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. Macrocyt formation from pairings of four different mating types in the subtropical form of *Dictyostelium purpureum*.

	S1 JKS 274-2	S2 JKS 275	S3 CUH 96	S4 M 97
S1 JKS 274-2	–	+	+	+
S2 JKS 275	+	–	+	+
S3 CUH 96	+	+	–	+
S4 M 97	+	+	+	–

Mating type S1: JKS 50, JKS 274-2. S2: JKS 275, KMM 1. S3: CUH 96, CUH 167. S4: M 97.

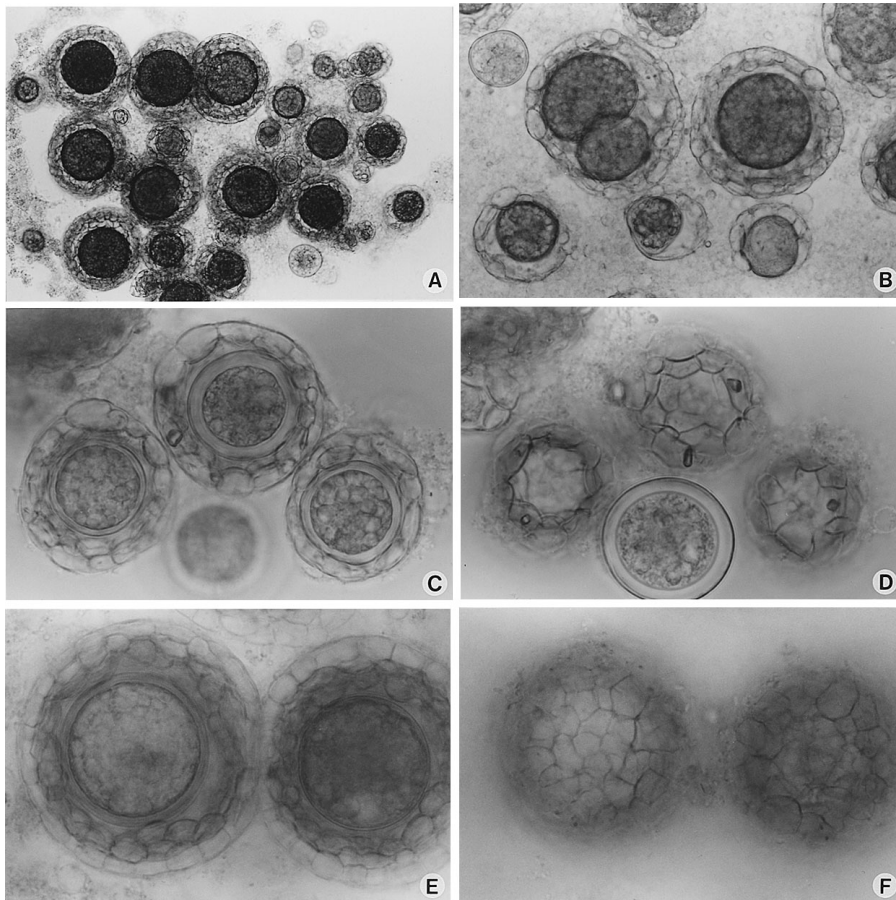


Fig. 1. Macrocyts of the mating group newly found in the subtropical form of *Dictyostelium purpureum*. A. Mass of macrocyts.  $\times 115$ . B. Macrocyts different in morphology and developmental stages.  $\times 460$ . C–F. Macrocyts. Note their outermost walls consisted by many vacuolated cells. Figs. D and F show the surfaces of the macrocyts in Figs. C and E, respectively.  $\times 460$ . 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).

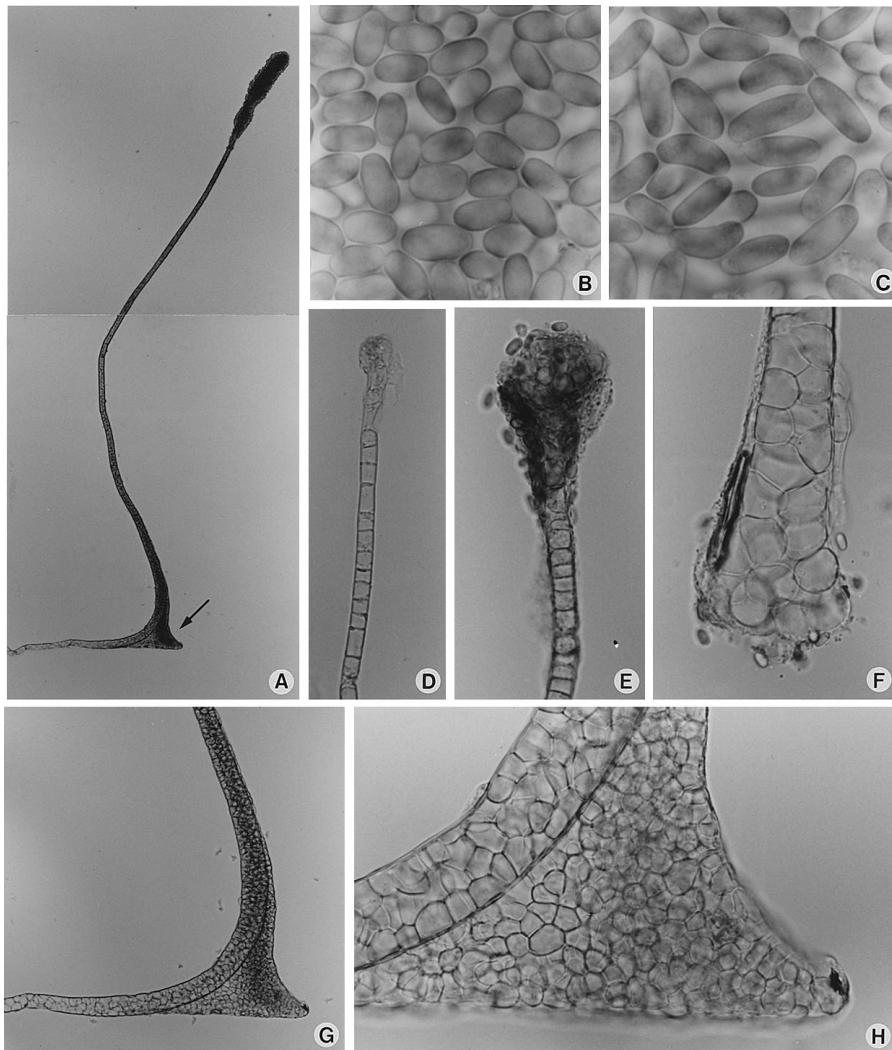


Fig. 2. A mating group newly found in the subtropical form of *Dictyostelium purpureum*. A. Upright part of a prostrate sorophore. Note a well-developed supporter indicated with an arrow.  $\times 45$ . B. Spores.  $\times 1150$ . C. Abnormal spores.  $\times 1150$ . D, E. Sorophore tips.  $\times 460$ . F. Sorophore base expanding conically or with a small disk.  $\times 460$ . G and H. Higher magnifications of the well-developed supporter indicated with an arrow in Fig. A. Fig. G,  $\times 115$ ; Fig. H,  $\times 460$ . 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.

Macrocyts 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 macrocyts (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 macrocyts were usually globose, mostly 19–57  $\mu\text{m}$  in diam (Min.: 12  $\mu\text{m}$ . Max.: 68  $\mu\text{m}$ . Range of the mean diameter: 29–44  $\mu\text{m}$ ). In these dimension values, the macrocyts of the subtropical form were not distinguishable from those of the temperate form.

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