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Taxonomic Studies on Dictyostelids. 3. Sexuality of the *Polysphondylium candidum* Complex

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Abstract A term "*Polysphondylium candidum* complex" was applied to the *Polysphondylium* strains bearing large spores and exhibiting elongation of sorophores. Macrocyst formation was examined in 27 strains of this complex. Based on the results, the strains were classified into 3 groups depending on their sexuality. (1) The heterothallic group, comprising 19 strains and including 2 mating types; the strains were confirmed as *P. candidum*. (2) The non-homothallic strain SH1, the *P. filamentosum* type strain that was distinctly distinguished from *P. candidum* in the mating system. (3) The homothallic group, comprising 9 strains. To determine whether this group can be accommodated in a single undescribed species, a further study on its morphology is required.
Key words: cellular slime molds, dictyostelids, macrocysts, *Polysphondylium*, *P. candidum*, *P. filamentosum*, sexuality.

Introduction

Polysphondylium candidum Hagiwara was originally described based on the isolates obtained from the cool temperate area of Japan, and it was primarily distinguished from other known white species of *Polysphondylium* by its large spores (Hagiwara, 1973). The original description states that at maturity, some sorocarps bear a sorus at the tip of a sorophore; in some, the sorophores elongate and eventually bear a smaller sorus at the tip; while in others, the sorophores greatly elongate and do not bear a sorus at the tip. Such elongation of sorophores is unique to *P. candidum* and has not been observed in other *Polysphondylium* species, although among dictyostelids or cellular slime molds, elongation had been reported in *Dictyostelium giganteum* Singh (Singh, 1947). After several years, elongation of sorophores was discovered in a new species of *Polysphondylium* — *P. pseudo-candidum* Hagiwara — obtained from the subtropical area of Japan (Hagiwara, 1979). This species was, however, distinctly different from *P. candidum* in that it bore small spores. In *Polysphondylium*, the third species showing sorophore elongation was *P. filamentosum* Traub, Hohl, & Cavender; this species was obtained from Switzerland (Traub *et al*., 1981). Besides sorophore elongation, *P. filamentosum* has another unique characteristic: typically, its branch not only bears a whorl of secondary branches (Raper, 1984) but also elongates like a sorophore (Traub *et al*., 1981). Judging from the original description of *P. filamentosum*, this species produces large spores identical to *P. candidum*. Moreover, both these species occur in the same areas in Japan (Cavender and Kawabe, 1989) and Germany (Cavender *et al*., 1995). Furthermore, both the characteristics of rebranching and branch elongation, which distinguish *P. filamentosum* from *P. candidum*, were not always observed even in the same strain cultured on non-nutrient agar (Hagiwara and Hosono, 2006). Therefore, it is possible that some strains of *P. filamentosum* have been misidentified as *P. candidum*, and *vice versa*. In addition, it is possible that unknown species closely resembling *P. candidum* and *P. filamentosum* in morphology have been misidentified as either of these 2 species. Considering

these possibilities, I have coined a term "*Polysphondylium candidum* complex" for the *Polysphondylium* species group bearing large spores and exhibiting sorophore elongation.

In the course of my study on Japanese dictyostelids, the strains of the *P. candidum* complex that produced a whorl of secondary branches were identified with *P. filamentosum* (Hagiwara and Hosono, 2006), while other strains of the complex were considered to belong to *P. candidum* (Hagiwara, 1976, 1984, 1988, 1995, 1998, 2000; Hagiwara and Kawakami, 2000, 2002; Kawakami and Hagiwara, 1999). Macrocyst formation test revealed that a few strains of *P. candidum* produced macrocysts independently, i.e., they were self-compatible. This finding suggests that *P. candidum* is homothallic in sexuality (Kawakami and Hagiwara, 1999).

Recently, I had an opportunity to study the dictyostelids on Yakushima Island located in southern Japan. Two homothallic strains obtained from the island were identified with *P. candidum* because of their large spores and elongation of sorophores. However, their distribution in the warm temperate area, somewhat delicate sorocarps, and smaller spores than those in the original description of *P. candidum* questioned the categorization of these homothallic strains under *P. candidum*. To resolve this doubt, I attempted to examine the sexuality of the strains in the *P. candidum* complex in the present study.

Materials and Methods

Twenty-seven strains of the *P. candidum* complex were used in this study (Table 1). These included 10 strains of *P. candidum*, including the type strain *Hagiwara* 168; 4 strains of *P. filamentosum*, including the type strain SH1; and 13 unidentified strains. All the strains were maintained at 20°C on non-nutrient agar with *Escherichia coli* (Migula) Castellani & Chalmers as the food bacterium.

Macrocyst formation was tested according to the procedure described by Kawakami and Hagiwara (1999). To test the mating competence, spores of each pair of strains were inoculated into small colonies of *E. coli* on 0.1 LP (0.1% lactose+0.1% proteose peptone) agar plates. For underwater cultures, 5 ml of sterile Bonner's salt solution (Bonner, 1947) was added to each plate after the spores had germinated. Cultures were incubated at 25°C in the dark and observed after 3 weeks of incubation.

Macrocysts of the dictyostelids have 3 surrounding walls: the primary outermost wall is fragile and fugacious, the secondary wall is rigid and persistent, and the tertiary wall is trilaminar and pliable (Erdos *et al*., 1972). Because the secondary wall is rigid and persistent, its outside diameter was used for measuring the macrocyst dimension. The macrocysts were mounted in distilled water, and their dimensions were measured using a Kogaku digital micrometer (×1000).

Results and Discussion

The 27 strains of the *P. candidum* complex were examined for their sexuality. All the strains, excluding the SH1 strain, were classified into 2 groups: the homothallic group and the heterothallic group. The homothallic group included 9 strains, namely, Ch17, JKS57, JKS124, JKS143, JKS215, KKY1, KKY6, TCK95 and TI123. The remaining 17 strains were assigned to the heterothallic group that was subdivided into 2 mating types, tentatively designated as *mt*1 and *mt*2. The results of the macrocyst formation test in all the non-homothallic strains paired with each of the 2 tester strains, NYA50 (*mt*1) and HdTk18-1 (*mt*2), are shown in Table 2.

Hagiwara 168, the type strain of *P. candidum*, was included in the heterothallic group. Therefore, this group was identified with *P. candidum*. CSB6, NYA55 and NYA65 had been previously identified with *P. filamentosum* (Hagiwara and Hosono, 2006) (Table 1); however, in the present study, not only none of them mated with the type strain of *P. filamentosum*, but also every strain mated with either of the 2 tester strains of *P. candidum* (Table 2). Consequently, we believed that these 3 strains should be accommodated in *P.*

Table 1. Strains of the *P. candidum* complex examined in this study.

Strain	Data of isolation	Reference
P. candidum		
*Hagiwara*168 (Type)	Japan, Tottori Pref., Mt. Daisen, 1971, by HH*	Hagiwara (1973)
Ch17	Japan, Akita Pref., Mt. Chokai, 1983, by HH	Hagiwara (1984)
IK53	Japan, Ibaraki Pref., Kashima-jingu, 1994, by SK	Kawakami and Hagiwara (1999)
JKS57	Japan, Wakayama Pref., Kada, 1998, by HH	Hagiwara (2000)
JKS124	Japan, Ehime Pref., Is. Ohmishima, 1998, by HH	Hagiwara (2000)
JKS143	Japan, Yamaguchi Pref., Ohata-cho, 1998, by HH	Hagiwara (2000)
JKS194	Japan, Okayama Pref., Mt. Gagyu-san, 1998, by HH	Hagiwara (2000)
JKS215	Japan, Hyogo Pref., Mt. Rokko-san, 1998, by HH	Hagiwara (2000)
TCK95	Japan, Tokyo Pref., the Imperial Palace, 1998, by HH	Hagiwara and Kawakami (2000)
TI123	Japan, Tokyo Pref., Is. Hachijo, 2002, by HH	Hagiwara and Kawakami (2002)
P. filamentosum		
SH1 (Type)	Switzerland, Osterfingen SH, 1970, by F. Traub	Traub *et al*. (1981)
CSB6	Japan, Chiba Pref., Boso-fudoki-no-oka, 1996, by HH	Hagiwara and Hosono (2006)
NYA55	Japan, Nagano Pref., the Yatsugatake Mts., 2005, by HH	Hagiwara and Hosono (2006)
NYA65	Japan, Nagano Pref., the Yatsugatake Mts., 2005, by HH	Hagiwara and Hosono (2006)
Unidentified		
EhI7-2	Japan, Ehime Pref., Mt. Ishizuchi, 1997, by SK**	
EhI9-1	Japan, Ehime Pref., Mt. Ishizuchi, 1997, by SK	
EhI13	Japan, Ehime Pref., Mt. Ishizuchi, 1997, by SK	
HdHn5	Japan, Hokkaido Pref., Nopporo Forest Park, 1998, by SK	
HdHn20	Japan, Hokkaido Pref., Nopporo Forest Park, 1998, by SK	
HdHn22-2	Japan, Hokkaido Pref., Nopporo Forest Park, 1998, by SK	
HdKn13-2	Japan, Hokkaido Pref., Nukabira, 1998, by SK	
HdSm15-2	Japan, Hokkaido Pref., Sapporo city, 1998, by SK	
HdTk16-1	Japan, Hokkaido Pref., Lake Kussharo, 1998, by SK	
HdTk18-1	Japan, Hokkaido Pref., Lake Kussharo, 1998, by SK	
KKY1	Japan, Kagoshima Pref., Is. Yaku, 2006, by HH	
KKY6	Japan, Kagoshima Pref., Is. Yaku, 2006, by HH	
NYA50	Japan, Nagano Pref., the Yatsugatake Mts., 2005, by HH	

* H. Hagiwara. ** S. Kawakami.

candidum.

IK53 listed in Table 1 as *P. candidum* had been previously tested for macrocyst formation and was found to be neither cross-compatible nor self-compatible (Kawakami and Hagiwara, 1999). In the present study, however, it was confirmed that the mating type of IK53 was the *mt*2 of *P. candidum* (Table 2). On the other hand, the Ch17, JKS57, JKS124, JKS143, JKS215, TCK95 and TI123 strains, listed together with IK53 in Table 1 as *P. candidum*, were found to be self-compatible and belonged to the homothallic group.

According to the biological species concept, the homothallic group of the *P. candidum* complex belongs to neither *P. candidum* nor *P. filamentosum* because of its different sexuality. To determine whether all the 9 strains of the homothallic group can be accommodated in a single undescribed species, a further detailed study on their morphology is required.

Although SH1, the type strain of *P. filamentosum*, was not homothallic, it could not mate with each of the 2 tester strains of *P. candidum* (Table 2). This observation suggests that *P. filamentosum* is distinctly different from *P. candidum* in the mating system and is a biologically distinct species.

Traub *et al*. (1981) stated that the distinguishing characteristics of *P. filamentosum*, such as the elongation of branches, were accentuated on 0.1 LP agar. On the other hand, rebranching and elongation of the branches were not observed in SH1, the type strain of *P. filamentosum*, when cultured on non-nutrient agar (Hagiwara and Hosono, 2006). In the present study, SH1

Table 2. Results of the macrocyst formation test in all the examined non-homothallic strains of the *P. candidum* complex.

	*mt*1 NYA50	*mt*2 HdTk18-1
P. candidum		
*Hagiwara*168 (Type)	−	+
EHI7-2	−	+
EHI9-1	−	+
EHI13	−	+
HdHn5	−	+
HdKn13-2	−	+
HdSm15-2	−	+
HdTk16-1	−	+
JKS194	−	+
NYA50	−	+
NYA55*	−	+
CSB6*	+	−
HdHn20	+	−
HdHn22-2	+	−
HdTk18-1	+	−
IK53	+	−
NYA65*	+	−
P. filamentosum		
SH1 (Type)	−	−

* These 3 strains had been identified with *P. filamentosum* in Hagiwara & Hosono (2006). See Table 1.

produced typical sorocarps on non-nutrient agar containing activated charcoal, although it produced abnormal sorocarps on 0.1 LP agar. It is known that the addition of activated charcoal, as a powder or small granules, to cultures at the time of cell aggregation enhances sorocarp formation (Raper, 1984). The addition of activated charcoal will be an effective method for obtaining accurately identified strains in order to discover the mating system of *P. filamentosum* in future.

At maturity, the macrocysts of *P. candidum* have an undulate tertiary wall (Fig. 1). Such a structure has been observed in *P. violaceum* Brefeld (Erdos *et al*., 1972), *P. pallidum* Olive and *P. tenuissimum* Hagiwara (Hagiwara, 1989), and *Dictyostelium monochasioides* Hagiwara and *P. pseudo-candidum* (Kawakami and Hagiwara, 1999). In *P. candidum*, the average diameter of the macrocysts was 28.4 μm (SD=5.8, n=210).

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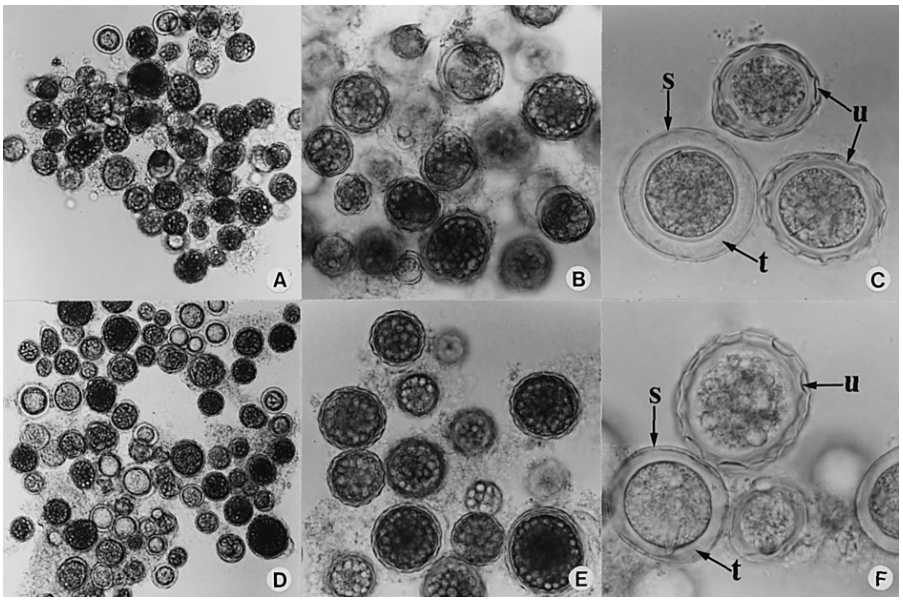

Fig. 1. Macrocysts of *P. candidum*. **A**–**C**. Macrocysts produced by pairing 2 compatible mating types, namely tester strains NYA50 (*mt*1) and HdTk18-1 (*mt*2). **D**–**F**. Macrocysts produced by pairing 2 compatible mating types, namely, strains HdHn5 (*mt*1) and NYA65 (*mt*2). Abbreviations: s, secondary wall; t, tertiary wall; u, undulate tertiary wall. **A** & **D**, ×115; **B** & **E**, ×230; and **C** & **F**, ×460.

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