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FULL PAPER

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Two mating groups of *Polysphondylium pallidum*, a dictyostelid cellular slime mold

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Abstract Seven strains representative of *Polysphondylium pallidum* were examined in terms of mating relationship and morphology. By mating tests, two heterothallic mating groups were detected. By morphological comparisons, they were clearly different in number of branches and in shape of bases and tip cells of sorophores. These results suggested that these two mating groups were distinct taxa.

Key words Dictyostelid cellular slime mold · Mating · *Polysphondylium pallidum* · Species concept · Taxonomy

Introduction

Polysphondylium Brefeld, a genus of the dictyostelid cellular slime molds, was founded on *P. violaceum* Brefeld. This genus was characterized by sorocarps with whorls of branches (Brefeld 1884). *P. pallidum* Olive was originally described as the second *Polysphondylium* species that had white sorocarps (Olive 1901). After more than 80 years passed, Raper (1984) redescribed it in detail, and Hagiwara (1989) also did so on the basis of Japanese isolates of one mating group. This species has been considered to be cosmopolitan and to be one of the dictyostelids characteristic of temperate biomes and deciduous forest soils (Swanson et al. 1999); it has, then, been often used in some biological studies (Table 1).

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We have considered that it is significant to introduce the biological species concept to the taxonomy of dictyostelids because their morphology is relatively simple and variable (Kawakami and Hagiwara 1999). Francis (1975, 1980) first discovered the macrocysts of *P. pallidum* that were formed during the sexual cycle and showed that genetic exchange occurred in its macrocysts. This fact suggests the possibility of using the biological species concept for taxonomic treatment of *P. pallidum*. In this species, two syngens, or mating groups, have already been discovered in eastern North America (Eisenberg and Francis 1977), and also one in Japan (Hagiwara 1995), although their morphological data were not sufficiently presented.

In the present article, we have selected seven strains of *P. pallidum* that are important in taxonomic study or other research subjects and examined these strains in detail on mating relationship and morphological comparisons to confirm the species concept of *P. pallidum*.

Materials and methods

Strains

Seven strains of *Polysphondylium pallidum* were used for mating tests and morphological comparisons (see Table 1). PN500 is an opposite mating type strain of PN600 (Francis 1975). Three strains, V-1, WS320, and WS543, have been considered as representing a species concept of *P. pallidum* by Raper and his colleagues (Raper 1984). CK8 is an opposite mating type strain of CK9 (Mizutani et al. 1990), and both of them represent Hagiwara's species concept of *P. pallidum* (Hagiwara 1989).

Mating tests

According to the procedures described by Kawakami and Hagiwara (1999), mating tests were performed as follows. Spores of each pair of strains were inoculated into small

Table 1. Seven strains representative of *Polysphondylium pallidum* examined in this study

Strain	Geographic origin	Source[a]	Research example
PN500	Delaware, USA	Univ. of Tsukuba	Macrocyst genetics (Francis 1980); whorl morphogenesis (Gregg et al. 1996)
PN600	Delaware, USA	Univ. of Tsukuba	Macrocyst genetics (Francis 1980)
V-1	Virginia, USA	KBR	Taxonomy (Raper 1984)
WS320	Madras, India	KBR	Axenic culture (Hohl and Raper 1963); cell–cell adhesion (Ochiai et al. 1997); microcyst germination (Hohl et al. 1970); taxonomy (Raper 1984)
WS543	Wisconsin, USA	KBR	Taxonomy (Raper 1984)
CK8	Chiba, Japan	TNS	Killer factor (Mizutani et al. 1990); taxonomy (Hagiwara 1989)
CK9	Chiba, Japan	TNS	Killer factor (Mizutani et al. 1990); taxonomy (Hagiwara 1989)

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colonies (~0.1 ml suspension poured) of *Escherichia coli* (Migula) Castellani et Chalmers as food on 0.1% lactose/proteose peptone agar plates (6 cm in diameter). After spores germinated, 6 ml sterile Bonner's salt solution (Bonner 1947) was poured into each plate for submerged culture. Plates were incubated at 25°C in the dark and observed after 3–4 weeks to judge whether macrocysts were present in the plates.

Morphological terminology of sorocarps

The terms used for the sorocarps of *Polysphondylium* followed Harper (1929) and Hagiwara (1989). "Terminal sorus" is a spore mass formed on a sorophore tip, and "lateral sorus" is a spore mass on a branch tip. "Node" is a point on a sorophore from which a whorl of branches originates. "Internode segment" is a portion between two adjacent nodes, and "terminal segment" is a portion between an uppermost node and a top of sorophore. In the present article, a terminal cell of a sorophore tip is named "tip cell" for the first time. In addition, we introduce the term "whorl index," which represents the ratio of the number of whorls with four or more branches to 40 whorls.

Morphological observations

Procedures of morphological observations mainly followed Hagiwara (1989). Spores were inoculated on ~1.7% nonnutrient agar plates. One drop (~0.1 ml) of *E. coli* suspension was put on the inoculated point of each plate, and these plates were incubated at 20°C for 7–10 days. As a rule, a granular type of activated charcoal was added to each plate, because it has been shown that this is beneficial for sorocarp formation in some dictyostelids (Raper 1984).

The number of whorls per sorocarp and branches per whorl were counted under a dissecting microscope. We did not regard a single branch as a regular one, i.e., a whorl. Sori were measured in diameter under an inverted microscope (×40) after 1 week cultivation. Bases of both sorophores and branches were measured in width under a microscope (×400). Internode segments, terminal segments, and branches were measured in length as follows: these parts were drawn as lines with an Abbe camera lucida attached to a microscope (×400, ×200, or ×100), and then the lines were measured with a rule. Spores were measured in size with a micrometer attached to a microscope (×1000).

Table 2. Result of pairings in all combinations among seven strains representative of *Polysphondylium pallidum*

Strain	PN500	PN600	V-1	WS543	CK8	CK9	WS320
PN500	−	+	+	−	−	−	−
PN600		−	−	+	−	−	−
V-1			−	+	−	−	−
WS543				−	−	−	−
CK8					−	+	−
CK9						−	−
WS320							−

Two squares represent different bundles of mating relationships

Results

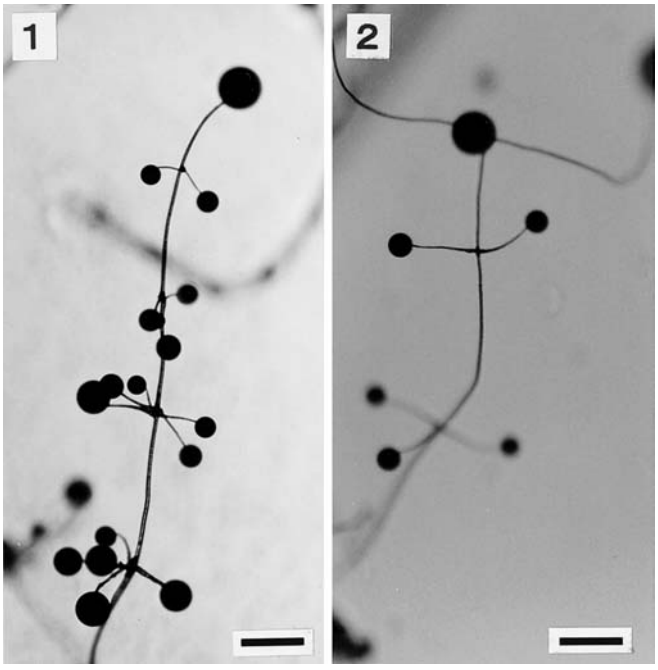
Mating

As a result of pairing in all the combinations of seven strains examined (Table 2), both PN500 and WS543 formed macrocysts with PN600 and V-1, and CK8 produced macrocysts only with CK9. However, WS320 did not form macrocysts with any strains. This result showed the existence of two heterothallic mating groups in *P. pallidum*. These two mating groups were tentatively named group A and B; the former included four strains, PN500, PN600, V-1, and WS543, and the latter two strains, CK8 and CK9.

Morphological comparisons

Group A and B developed a maximum of 14 and 11 whorls per sorocarp, respectively. Group A had a tendency to produce a larger number of branches per whorl than group B (Figs. 1, 2). The mean values of branches per whorl of six strains were as follows: PN500, 5.1 ± 1.4 (n = 76); PN600, 4.7 ± 1.0 (n = 104); V-1, 4.4 ± 1.0 (n = 67); WS543, 4.1 ± 1.0 (n = 74); CK8, 3.5 ± 0.7 (n = 45); CK9, 3.0 ± 0.9 (n = 42). This tendency became marked by calculating each whorl index (Fig. 3). The index values of group A were larger than 0.7; those of group B were 0.5 or less than 0.5.

The terminal sori of group A and B were 50–170 μm and 70–240 μm in diameter, respectively; the lateral sori of group A and B were 20–110 μm and 30–150 μm in diameter, respectively. Group B showed a tendency to produce larger sori, but this tendency depended on the sorus size of one strain of group B, CK8 (terminal sori, 100–240 μm; lateral sori, 40–150 μm). The sorus size of CK9 or the other strain of group B (terminal sori, 70–160 μm; lateral sori, 30–90 μm) was included in that of group A.



Figs. 1,2. Sorocarps of two mating groups of *Polysphondylium pallidum*. **1** Group A (PN500). **2** Group B (CK8). *Bars* 200 μm

The bases of prostrate sorophores of two groups tended to be acuminate. However, those of the nonprostrate sorophores of group A were usually round (Fig. 4), sometimes clavate or conical, whereas those of group B were clavate (Fig. 5). The sorophore bases of group A were thicker than those of group B (Table 3). The sorophore tips of two groups were acuminate (Figs. 6, 7), although those of group B were sometimes piriform. The sorophore tips of group A were thicker than those of group B (Table 3). The tip cells of group A were ovoid (Fig. 6), while those of group B were subulate (Fig. 7).

The internode segments of group A were somewhat shorter than those of group B (Table 3). The terminal segments of group A mostly overlapped in length with those of group B, although those of two strains, V-1 of group A and CK9 of group B, were longer than those of the others (Table 3). Also, the terminal segments of three strains, V-1, WS543, and CK9, sometimes exceeded two times the average length (data not shown).

Both groups had branches with clavate bases and acuminate tips, but group A showed a tendency to have shorter branches with thinner bases and thicker tips (Table 4).

Both groups had oblong to elliptical spores with unconsolidated polar granules. The spores of group A were also similar in size and mean length:width ratio to those of group B (Table 5), although those of PN500 were especially smaller in ratio than those of the other strains of group A.

Discussion

Two heterothallic mating groups were recognized in seven strains representative of *P. pallidum* and were tentatively named group A and B. It is known the progenies had hatched off from the macrocysts formed between PN500

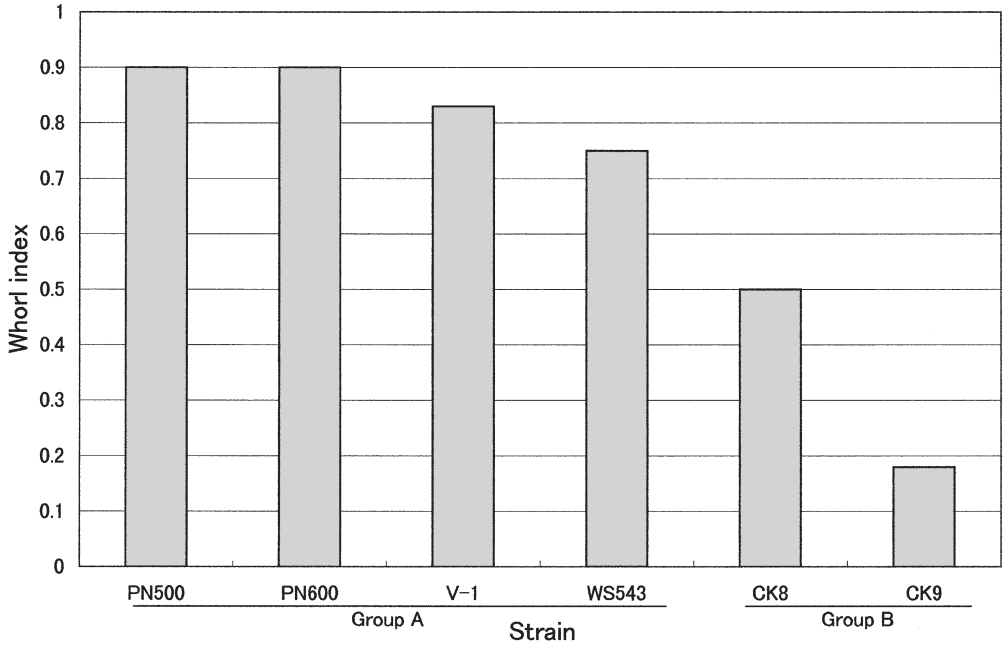
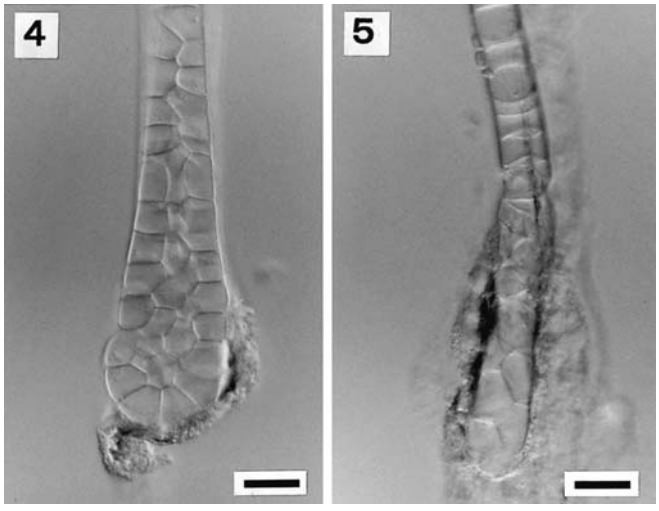


Fig. 3. Comparison of whorl index between two mating groups of *Polysphondylium pallidum*

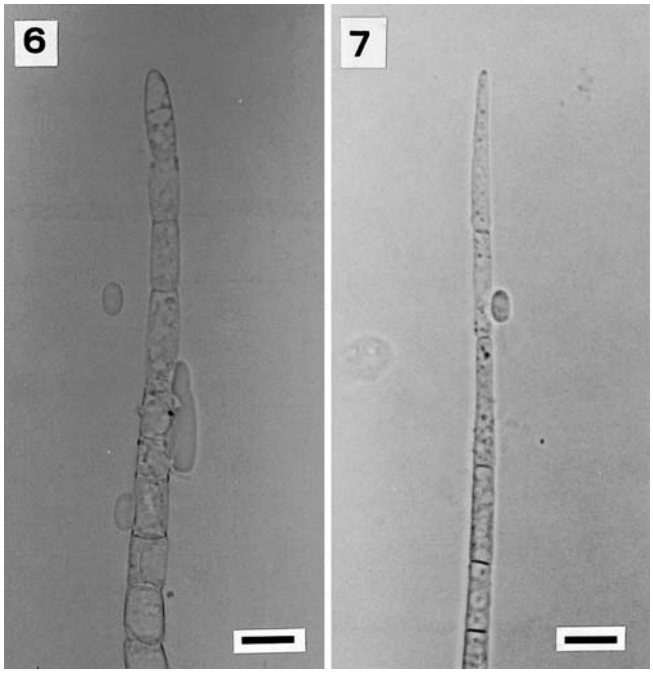
and PN600 (Francis 1975, 1980). Therefore, it is possible to apply the biological species concept to two groups.

Groups A and B were compared on several macroscopic and microscopic characters. Most quantitative data overlapped between two groups. Moreover, the coefficients of variation of the quantitative characters examined were relatively high (mostly 20%–40%), except spore size (less than 13.1%). This result agrees with previous data on *Polysphondylium* (Hagiwara 1989). The sori of CK9 were smaller than Hagiwara's description (150–260 μm) of *P. pallidum*, including the data of CK9 (Hagiwara 1989). The difference occurs because the diameter of mature sori is variable under the influence of moisture conditions, as previously pointed out (Hagiwara 1989).

However, the two groups are separable by comparison of some morphological characters. Group A has numerous



Figs. 4,5. Nonprostrate sorophore bases of two mating groups of *Polysphondylium pallidum*. **4** Group A (V-1). **5** Group B (CK8). *Bars* 20 μm



Figs. 6,7. Sorophore tips of two mating groups of *Polysphondylium pallidum*. **6** Group A (PN500). **7** Group B (CK8). *Bars* 10 μm

Table 3. Morphological comparison of sorophores between two mating groups of *Polysphondylium pallidum*

Group	Strain	Width of sorophore base (μm)[a]	Width of sorophore tip (μm)[b]	Length of internode segment (μm)	Length of terminal segment (μm)[c]
A	PN500	30.5 ± 6.4, $n = 19$	6.0 ± 1.8, $n = 11$	472 ± 95, $n = 37$	494 ± 140, $n = 8$
	PN600	30.4 ± 7.8, $n = 24$	4.6 ± 0.9, $n = 13$	493 ± 46, $n = 42$	418 ± 138, $n = 21$
	V-1	40.1 ± 4.4, $n = 9$	7.6 ± 1.9, $n = 11$	558 ± 74, $n = 16$	624 ± 177, $n = 9$
	WS543	26.8 ± 3.4, $n = 10$	5.6 ± 2.2, $n = 10$	572 ± 75, $n = 25$	413 ± 125, $n = 14$
B	CK8	19.2 ± 4.8, $n = 11$	3.3 ± 0.7, $n = 15$	640 ± 108, $n = 30$	543 ± 177, $n = 13$
	CK9	19.6 ± 4.3, $n = 10$	3.3 ± 1.1, $n = 10$	607 ± 108, $n = 27$	656 ± 279, $n = 6$

[a] Measured at the thickest part
[b] Measured at a level 50 μm below the top
[c] Lengthened segments were excluded from average length calculations

Table 4. Morphological comparison of branches between two mating groups of *Polysphondylium pallidum*

Group	Strain	Width of branch base (μm)[a]	Width of branch tip (μm)[b]	Branch length (μm)
A	PN500	8.1 ± 2.1, $n = 22$	4.1 ± 0.8, $n = 22$	168 ± 25, $n = 21$
	PN600	8.8 ± 1.7, $n = 12$	4.5 ± 0.3, $n = 12$	228 ± 31, $n = 24$
	V-1	11.5 ± 4.5, $n = 12$	5.0 ± 0.9, $n = 11$	197 ± 28, $n = 20$
	WS543	9.7 ± 2.0, $n = 8$	5.1 ± 1.2, $n = 8$	191 ± 31, $n = 25$
B	CK8	11.9 ± 2.7, $n = 25$	3.8 ± 0.8, $n = 20$	262 ± 69, $n = 25$
	CK9	14.6 ± 3.6, $n = 20$	3.5 ± 0.4, $n = 20$	254 ± 73, $n = 20$

[a] Measured at the thickest part
[b] Measured at a level 50 μm below the top

Table 5. Morphological comparison of spores between two mating groups of *Polysphondylium pallidum*

Group	Strain	Spore size (μm)[a]	Length/width ratio
A	PN500	6.3 ± 0.6 × 3.8 ± 0.4	1.64 ± 0.09
	PN600	6.6 ± 0.7 × 3.5 ± 0.3	1.89 ± 0.17
	V-1	6.7 ± 0.7 × 3.6 ± 0.4	1.90 ± 0.15
	WS543	7.0 ± 0.7 × 3.8 ± 0.5	1.84 ± 0.17
B	CK8	6.9 ± 0.5 × 4.0 ± 0.3	1.74 ± 0.10
	CK9	7.0 ± 0.4 × 4.0 ± 0.4	1.77 ± 0.10

[a] Twenty spores were measured in diameter on six strains

branches, and the sorophores have round bases and ovoid tip cells. In contrast, group B is characterized by fewer branches per whorl and the clavate bases and subulate tip cells of the sorophores. Moreover, group B has longer internode segments and longer branches with relatively thick bases and thin tips. This morphological separation between groups A and B leads to the suggestion that the two mating groups are clearly distinct taxa. Hereafter, it is necessary to examine these two presumed taxa more in detail and give them taxonomic positions.

WS320 may be asexual or belong to another mating group because this strain did not produce macrocysts with any strains. WS320 is also different from two mating groups in having fewer whorls (a maximum of four) per sorocarp. In addition, it is characterized by clavate bases and ovoid tip cells of sorophores. Therefore, it is possible that WS320 belongs to another taxon.

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