

SPORANGIOSPORE VARIABILITY IN *PILOBOLUS*

K. MICHAEL FOOS and BRENDA S. JEFFRIES

Department of Biology
Indiana University East
Richmond, Indiana 47374

INTRODUCTION

Sporangiospore size and shape are taxonomic characteristics given for all species of *Pilobolus* (Grove, 1934; Zycha, *et al.*, 1969). To be of taxonomic value, spore size and shape must remain consistent. While it is widely held that these characteristics are consistent, no records exist in the literature to indicate that this is the case over time and through subsequent transfers. Recently, variability was observed in spore size and shape during the first few days after transfer, suggesting that these characteristics might be of questionable taxonomic usefulness. This project was designed to determine if sporangiospore size and shape remain consistent enough over time and through subsequent transfers to justify their use as dependable taxonomic characteristics.

METHODS AND MATERIALS

Two isolates of *Pilobolus* were recovered from domestic animals in Fayette County, Indiana, using the technique of Foos and Royer (1986). *Pilobolus longipes* was recovered from horse feces and *P. crystallinus* from cow feces. Single sporangia, collected from the original fecal samples, were transferred to microscope slides in a drop of sterile distilled water and carefully disarticulated using a sterile probe. Spores from these sporangia were examined with a brightfield microscope and then transferred to petri dishes containing dung agar to provide cultures for subsequent transfers through several sporulation cycles. All cultures were maintained on dung agar at room temperature (20°C) under cool white fluorescent lights with an intensity of 2000 lx and 12 hr alternating light and dark periods.

Two methods of successive transfers were compared. In one set of experiments, sporangia produced on cultures were collected and transferred to petri dishes containing fresh media. In the second set of experiments, cores of agar containing hyphal tips were cut from the growing mycelia with a sterile 8 mm cork bore and transferred to petri dishes containing fresh media.

From each subsequent culture, sporangia containing sporangiospores were collected daily for the first four days after the initiation of sporulation. These sporangia were mounted in lactophenol on microscope slides and examined with a 1000X brightfield microscope. Spore dimensions of 50 sporangiospores per sporangium were measured and recorded. This procedure was repeated after each subsequent transfer for 6 or 11 transfers. Spore size measurements were compared and data analyzed using Minitab statistical software.

RESULTS

Sporangiospores of *P. longipes* had an overall mean length of $11.0 \pm 0.8 \mu\text{m}$ and an overall mean width of $10.2 \pm 0.8 \mu\text{m}$. The mean dimensions of the spores found growing on the original cultures collected from dung were 12.5 ± 0.3 by

TABLE 1. Dimensions (μm) and length to width ratios of sporangiospores of *P. longipes* and *P. crystallinus* collected daily for the first four days after the initiation of sporulation.

Day	<i>P. longipes</i>			<i>P. crystallinus</i>		
	Length	Width	Ratio	Length	Width	Ratio
1	11.0 $\pm$ 0.7 ^a	10.2 $\pm$ 0.7	1.08	9.1 $\pm$ 0.9	5.0 $\pm$ 0.5	1.55
2	10.7 $\pm$ 0.9	9.8 $\pm$ 0.9	1.08	9.4 $\pm$ 0.4	6.0 $\pm$ 0.3	1.55
3	10.9 $\pm$ 0.7	10.0 $\pm$ 0.6	1.09	9.1 $\pm$ 0.5	5.8 $\pm$ 0.3	1.57
4	11.0 $\pm$ 1.0	10.2 $\pm$ 0.9	1.08	9.3 $\pm$ 0.4	6.0 $\pm$ 0.4	1.54

^aStandard deviation

11.5 $\pm$ 0.4 μm , while those from cultures produced by sporangia transferred to dung agar had mean dimensions of 10.6 $\pm$ 0.6 by 10.0 $\pm$ 0.6 μm , and those from cultures produced by hyphal tips transferred to dung agar had mean dimensions of 11.2 $\pm$ 0.9 by 10.4 $\pm$ 1.0 μm . Sporangiospores of *P. crystallinus* had an overall mean length of 9.3 $\pm$ 0.6 μm and an overall mean width of 5.9 $\pm$ 0.5 μm . The mean dimensions of the spores found growing on the original cultures collected from dung were 10.4 $\pm$ 0.5 by 7.2 $\pm$ 0.6 μm , while those from cultures produced by sporangia transferred to dung agar had mean dimensions of 9.3 $\pm$ 0.4 by 5.9 $\pm$ 0.5 μm , and those from cultures produced by hyphal tips transferred to dung agar had mean dimensions of 9.0 $\pm$ 0.4 by 5.9 $\pm$ 0.5 μm . Dimensions of sporangiospores produced during the first four days after the initiation of sporulation and those produced after different numbers of transfers are given in Tables 1 and 2.

Sporangiospore shape can be quantified by determining the length to width ratio of the spores. The overall mean length to width ratio of *P. longipes* spores was 1.08 $\pm$ 0.04. The mean length to width ratio of the original spores found growing on dung was 1.08 $\pm$ 0.02. Cultures growing on dung agar transferred by sporangia had a mean length to width ratio of 1.06 $\pm$ 0.03, while those growing on dung agar from hyphal tip transfers had a mean length to width ratio of 1.10 $\pm$ 0.04. *Pilobolus crystallinus* had an overall mean length to width ratio of 1.54

TABLE 2. Dimensions (μm) and length to width ratios of sporangiospores of *P. longipes* and *P. crystallinus* produced after each transfer.

Tr ^a	<i>P. longipes</i>			<i>P. crystallinus</i>		
	Length	Width	Ratio	Length	Width	Ratio
1	10.7 $\pm$ 0.5 ^b	9.8 $\pm$ 0.3	1.09	9.7 $\pm$ 0.2	6.0 $\pm$ 0.8	1.61
2	10.4 $\pm$ 0.6	9.7 $\pm$ 0.6	1.07	9.6 $\pm$ 0.2	5.8 $\pm$ 0.5	1.65
3	10.5 $\pm$ 0.3	9.6 $\pm$ 0.6	1.09	9.3 $\pm$ 0.4	6.1 $\pm$ 0.3	1.52
4	11.3 $\pm$ 0.9	10.4 $\pm$ 0.8	1.09	9.4 $\pm$ 0.5	6.4 $\pm$ 0.4	1.48
5	10.7 $\pm$ 0.6	9.8 $\pm$ 0.6	1.09	8.9 $\pm$ 0.6	5.9 $\pm$ 0.4	1.51
6	11.4 $\pm$ 0.8	10.7 $\pm$ 0.8	1.07	9.1 $\pm$ 0.8	5.7 $\pm$ 0.6	1.61
7				9.0 $\pm$ 0.8	5.7 $\pm$ 0.3	1.56
8				8.9 $\pm$ 0.9	5.7 $\pm$ 0.4	1.58
9				8.9 $\pm$ 0.8	5.8 $\pm$ 0.2	1.53
10				9.4 $\pm$ 0.8	5.8 $\pm$ 0.2	1.62
11				8.7 $\pm$ 0.4	6.0 $\pm$ 0.3	1.46

^a Number of transfers

^b Standard deviation

± 0.15 . The mean length to width ratio of the original spores found growing on dung was 1.45 ± 0.05 . Cultures growing on dung agar transferred by sporangia had a mean length to width ratio of 1.58 ± 0.12 , while those growing on dung agar from hyphal tip transfers had a mean length to width ratio of 1.54 ± 0.19 . Length to width ratios of sporangiospores produced during the first four days after the initiation of sporulation and those produced after different numbers of transfers are given in Tables 1 and 2.

DISCUSSION

Taxonomic descriptions of all species of *Pilobolus* contain observations about sporangiospore size and shape. Van Tieghem (1876) observed the spores of *P. longipes* to be 12-14 by 10-12 μm ; these measurements were repeated by Fischer (1892). Grove (1934) followed the original description closely, stating that the spores were globose or ovoid, 12-15 by 10-12 μm . Zycha *et al.* (1969) expanded these measurements, stating that the spores were oval to oblong, 7.4-16.8 by 7.2-14.9 μm . Spores from the authors; experiments fall within these ranges with a mean length of 11.0 (range 9.1 to 13.6) μm and a mean width of 10.2 (range 8.7 to 12.0) μm .

Spores of *P. crystallinus* were observed by van Tieghem (1876) to be 8-10 by 5-6 μm ; Fischer (1892) found the spores to be 5-10 by 3-6 μm . Grove (1934) stated that they were 5-10 by 4-6 μm . Zycha *et al.* (1969) observed that the spores were ellipsoidal, 5-12 by 3-7 μm . Spores from the authors; experiments fall within these ranges with a mean length of 9.3 (range 8.4 to 10.9) μm and a mean width of 5.9 (range 5.2 to 7.8) μm .

For both species, the ranges of spore sizes from the original descriptions and more recent observations give wide variations in sporangiospore sizes within species. Spores produced under all test conditions were within published taxonomic limits.

Sporangiospore sizes were remarkably consistent within a single sporangium. However, sporangiospores produced in different sporangia varied significantly in size from one sporangium to another, even when these sporangia developed from a single mycelium. This made it very difficult to compare sporangiospore sizes from sporangia produced on different days or after different numbers of transfers. The differences in sporangiospore sizes from different sporangia produced on a given day from a single mycelium were as great as those produced in different sporangia from subsequent transfers. Thus, it appears that there is no significant difference in sporangiospore sizes from cultures caused by time or number of transfers. In every case, the mean sporangiospore size from any sporangium was similar to that of all other mean sporangiospore sizes from that isolate. There was no pattern of sporangiospore size change over time or from subsequent transfers.

Describing the shape of sporangiospores by using the length to width ratio indicates overall sporangiospore shape without regard to the size of the spore. *Pilobolus longipes*, described as having globose, ovoid, or oblong spores had a length to width ratio of 1.08 ± 0.04 (range 1.01 to 1.19). No pattern of different ratios was seen on different days or after different numbers of transfers. *Pilobolus crystallinus*, described as having ellipsoid spores, had a length to width ratio of

1.57 ± 0.12 (range 1.38 to 1.81). Again, no pattern of different ratios was seen on different days or after different numbers of transfers.

No pattern of sporangiospore size or shape variation was seen in either *P. longipes* or *P. crystallinus* when transferred over a relatively long period of time. Individual sporangiospore size and shape fluctuations appeared between mean measurements of spores from different sporangia from different mycelia, but these were no greater than those from sporangia produced on the same mycelia. Mean sizes and shapes of sporangiospores from all sporangia were very similar and well within the ranges published in species descriptions. Variations in sporangiospore sizes and shapes from different sporangia were so small that these sizes and shapes may be used confidently in taxonomic descriptions.

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