# Dictyostelids in Japan. XII. *Dictyostelium gloeosporum*, a new species from the grounds of the Imperial Palace, Tokyo

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**Abstract** A new species of dictyostelids, *Dictyostelium gloeosporum*, is described on the basis of isolates of soil from the grounds of the Imperial Palace, Tokyo. This species is characterized by large sori disproportionate to short sorophores rarely exceeding 2 mm, clavate sorophore tips which are simple or partially compound, and very sticky ellipsoid spores without polar granules.

Key words: dictyostelid, Dictyostelium gloeosporum, Japan, macrocyst.

In the course of this study on Japanese dictyostelids, a new species of *Dictyostelium* was obtained from soils of the Fukiage Gardens in the Imperial Palace, Tokyo. Descriptions and observations of this species are presented below.

Procedures of isolation, cultivation and observation are the same as those reported previously (Hagiwara, 1989). Twenty spores per isolate are used for calculating the mean spore diameter. Range of mean spore diameters of the isolates examined is indicated by MD in the following description. Mating tests follow the procedures described by Kawakami & Hagiwara (1999). The type specimen is preserved in the herbarium of the Department of Botany, National Science Museum, Tokyo (TNS).

#### Dictyostelium gloeosporum Hagiwara, sp. nov.

In culturis ad 20°C in agaro sine nutrimento et *Escherichia coli* immixto, sorocarpiis plerumque solitariis, interdum gregariis, non ramosis, phototropicis, interdum prostratis; sorophoris hyalinis,  $(0.2-)0.7-1.9 \,\mathrm{mm}$  longis, e basi dilatata oriundis, sursum gradatim attenuatis, apice anguste clavatis, plerumque ex cellulis praeter basim et apicem uniseriatis constans, prope basin  $(3.5-)6-13 \,\mu\mathrm{m}$  crassis, ad apicem latissimum  $3.5-7.5 \,\mu\mathrm{m}$  crassis; soris albis, globosis,  $40-250 \,\mu\mathrm{m}$  in diam; sporis hyalinis, ellipsoideis, plerumque 1.3-1.6-

plo longioribus quam latioribus, levibus, plerumque  $4.6-6.4\times3.3-4.6\,\mu\text{m}$ , sine granulis polaribus; pseudoplasmodiis non vel indefinite radiatis, non moventibus ubi sorophorum non formantibus, plerumque sorocarpia singula producentibus.

When cultured at 20°C on non-nutrient agar with Escherichia coli, sorocarps usually solitary, sometimes gregarious (Figs. 2F, 2G & 2I), unbranched, phototropic, sometimes prostrate; colorless, sorophores  $(0.2-)0.7-1.9 \,\mathrm{mm}$ length, sometimes exceeding 2 mm if prostrate, gradually tapering from bases to tips, usually consisting of a single tier of cells except in both terminal parts; sorophore bases nearly conical (Figs. 1C & 3C),  $(3.5-)6-13 \mu m$  in diam at a level  $100 \,\mu m$  above the bottom; sorophore tips clavate, simple or partially somewhat compound (Figs. 1A & 3A),  $3.5-7.5 \mu m$  in diam at the thickest portion; sori white, globose,  $40-250 \mu m$ in diam; spores hyaline, ellipsoid (Fig. 3E), usually 1.3-1.6 times longer than broad, smooth, mostly 4.6–6.4×3.3–4.6 (MD 5.2–5.9×3.7–4.2)  $\mu$ m, without polar granules, but often with irregular granules; pseudoplasmodia with no or indefinitely radiate streams (Figs. 2A-2E & 2H), not migrating without sorophore formation, usually producing single sorogens.

Habitat: In humus and fermentation layers of

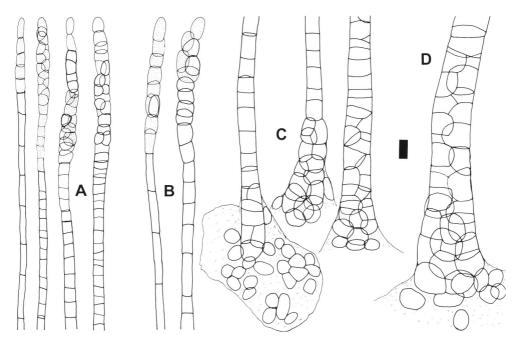


Fig. 1. Dictyostelium gloeosporum. A. Sorophore tips. B. Sorophore tips consisting of large-sized cells. C. Sorophore bases. D. Sorophore base consisting of large-sized cells. Bar= $10 \, \mu m$ .

soils in Tokyo, Japan.

Isolates examined: TCK40, from soil at 30–40 m alt. in the Fukiage Gardens of the Imperial Palace, Chiyoda-ku, Tokyo, Japan, 19 Nov. 1998, reported as "*Dictyostelium brefeldianum* variant" in Hagiwara & Kawakami (2000); TCK52 and TCK75, ibid., 17 Aug. 1999.

Type specimen: M-50026 (TNS), ex TCK52. Etymology: Greek, *gloeo*, in reference to compactly sticky spore mass of this species.

Hagiwara (1989) classified Japanese species of *Dictyostelium* into five categories based on the maximum length of non-prostrate sorophores. Namely, they are "minute species", "small species", "medial species", "large species" and "gigantic species", which do not or rarely produce sorophores exceeding 1 mm, 2 mm, 3.5 mm, 6 mm and 10 mm, respectively. According to his categories, *D. gloeosporum* is a small species. This species is macroscopically characterized by large sori disproportionate to short sorophores like *D. discoideum* Raper and *D. macrocephalum* Haigwara, Yeh & Chien. Sorocarps of *D. gloeosporum* grow close to one another. Howev-

er, this species is not "gregarious" but "solitary" in terms of growth habits (Hagiwara, 1989), because each sorocarp usually arises from a single pseudoplasmodium. The sorocarps usually stand erect or suberect. But, when cultured in one-sided light, they incline toward the light and their sori often touch the agar surface. Then spores of the collapsed sori germinate into myxamoebae, which also produce inclined secondary sorocarps. This process continues for two or three generations (Fig. 2J).

This new species is readily distinguishable from many other species by features of the spore. Its spores are so sticky that it is often difficult to remove them from a transplanting needle for microscopic observation. If successfully removed, most spores remain in a compacted mass and hardly disperse in the mount solution on the slide.

Dictyostelium gloeosporum is microscopically characterized by nearly simple and clavate sorophore tips and ellipsoid spores without polar granules. The spores often have many granules inside, which sometimes appear as unconsolidat-

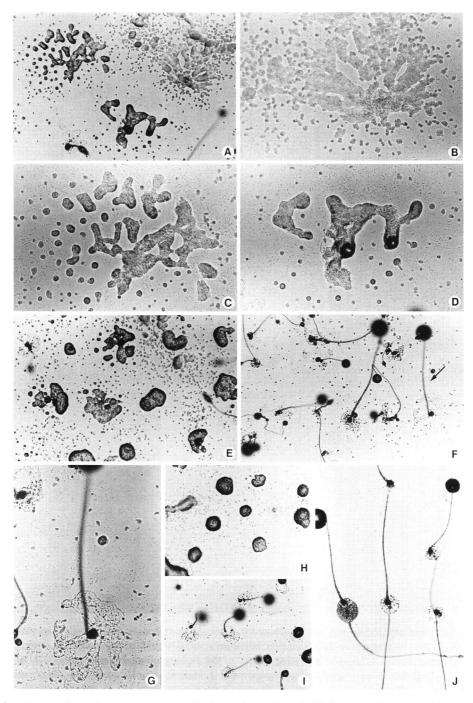


Fig. 2. Dictyostelium gloeosporum. A. Pseudoplasmodia. ×28. B–D. Higher magnifications of three pseudoplasmodia in Fig. A. Note that myxamoebae aggregate with indefinite streams. ×62. E. Irregular shaped pseudoplasmodia. ×28. F. Growth of sorocarps. One or two sorocarps formed from each pseudoplasmodium. ×28. G. Higher magnification of the sorocarp indicated by an arrow in Fig. F. This sorocarp formed from a somewhat large pseudoplasmodium. ×62. H. Mound-like pseudoplasmodia. ×28. I. Growth of sorocarps made from mound-like pseudoplasmodia. Each sorocarp grew from a single pseudoplasmodium. ×28. J. Growth of sorocarps under a one-sided light. Note that each secondary sorocarp was produced at a collapsed sorus. ×28. Figs. A–G & J, strain TCK52; Fig. H, strain TCK75; Fig. I, strain TCK40.

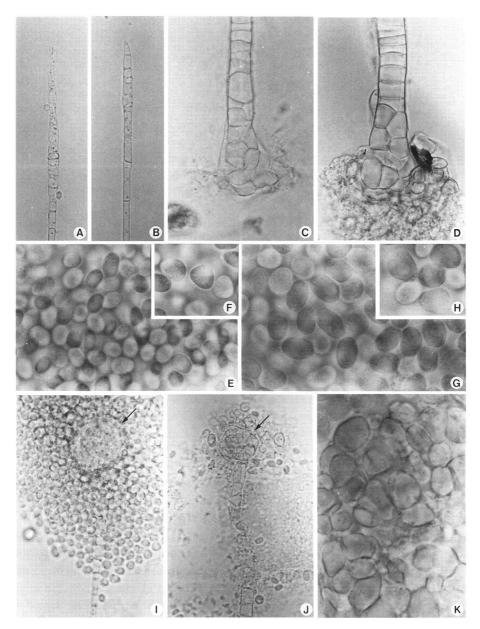


Fig. 3. Dictyostelium gloeosporum. A. Sorophore tip. ×460. B. Sorophore tip consisting of large-sized cells. ×460. C. Sorphore base. ×460. D. Sorophore base consisting of large-sized cells. ×460. E. Spores. ×1150. F. Abnormal spores. ×1150. G. Large-sized spores. ×1150. H. Large-sized abnormal spores. ×1150. I. Mass of vacuolated cells in a collapsed sorus (arrow). ×460. J. Mass of vacuolated cells in a sorus (arrow). The mass is surrounded by vacuolated cells, spores and empty spore cases. ×460. K. Higher magnification of a mass of vacuolated cells in a sorus. ×1150. Fig. A, TCK40; Figs. B, G–I & K, strain TCK3; Figs. C, E, F & J, strain TCK52; Fig. D, strain TCK20.

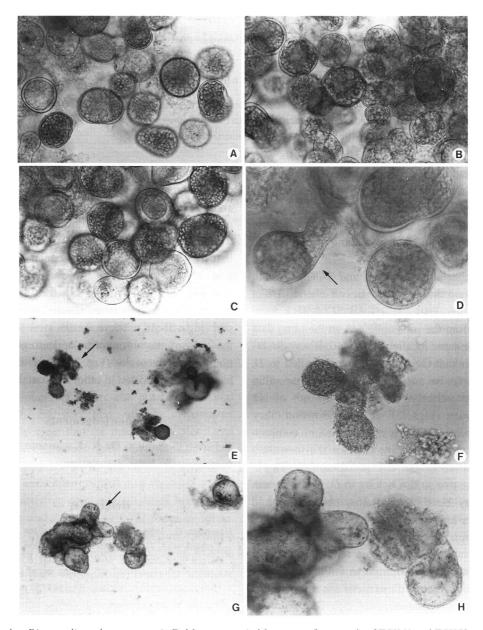


Fig. 4. *Dictyostelium gloeosporum*. A–D. Macrocysts. A. Macrocysts from a pair of TCK40 and TCK52. ×230. B. Macrocysts from a pair of TCK3 and TCK20. ×230. C. Macrocysts from a pair of TCK3 and TCK40. ×230. D. Irregular shaped macrocyst from a pair of TCK40 and TCK75 (arrow). ×460. E–H. Macrocyst-like bodies. Macrocyst-like bodies in Fig. F and Fig. H are higher magnifications of those in Fig. E and Fig. G (arrows), respectively. Figs. E & G, ×46; Figs. F & H, ×115. Figs. E & F, strain TCK20; Figs. G & H, strain TCK40.

ed polar granules. Abnormally large spores are rarely mixed with normal spores. These large spores are angular in shape (Fig. 3F), though abnormal spores in other dictyostelids are reniform or sigmoid in general (Hagiwara, 1989).

All pairs of the three strains examined were tested for formation of macrocysts characteristic of the sexual cycle. As a result, all strains were self-incompatible. TCK40 made macrocysts with TCK52 and TCK75, but a pair of the latter two made no macrocysts (Table 1). These facts suggest that *Dictyostelium gloeosporum* is heterothallic. Macrocysts of *D. gloeosporum* are usually spherical, but sometimes irregular in shape (Figs. 4A & 4D).

Two other strains, TCK3 and TCK20, which mostly fitted the characteristics of Dictyostelium gloeosporum (Figs. 1B, 1D, 3B, 3D & 3H), were isolated from the soils collected on the same date and in the same locality as TCK40. It was doubtful at first that these two strains belonged to D. gloeosporum because their spores were normal in shape but clearly larger than those of D. gloeosporum (Figs. 3E & 3G). However, they were shown to have a mating system common with D. gloeosporum by tests for the formation of macrocysts (Table 1, Figs. 3B & 3C). Therefore, these two strains belong to D. gloeosporum. They may be diploid strains, because diploid spores are known to be larger than haploid ones in D. discoideum (Sussman & Sussman, 1962).

TCK3 and TCK20 have a tendency to make larger sorocarps in addition to the larger spores. Therefore, sorocarp dimensions are not included in the above description of *Dictyostelium gloeosporum*. Their sorocarp dimensions were as follows: When cultured at 20°C on non-nutrient agar with *Escherichia coli*, sorophores were (0.2-)1.1-2.7 mm in length; sorophore bases were (4-)10-25  $\mu$ m in diam at a level 100  $\mu$ m above the bottom; sorophore tips were 4-14  $\mu$ m in diam at the thickest portion; sori were (30-)140-270  $\mu$ m in diam; spores were usually 1.3-1.6 times longer than broad, and mostly  $6.3-8.2\times4.3-5.5$  (MD  $6.9-7.4\times4.7-5.0$ )  $\mu$ m.

Table 1. Macrocyst formation among paired strains of *Dictyostelium gloeosporum*.

	TCK 3	TCK 20	TCK 40	TCK 52	TCK 75
TCK 3	_	+	+	_	_
TCK 20		_	1-	+	+
TCK 40			-	+	+
TCK 52				_	
TCK 75					_

It is noteworthy that all the strains examined produced macrocyst-like structures in underwater cultures (Figs. 4E–4H). These structures were superficially similar to pseudomacrocysts, which were named by Hagiwara (1984), but different in that they lacked spores and vacuolated cells inside.

Finally, all the strains except for TCK40 contained single masses of vacuolated cells in some sori (Figs. 3I–3K). These masses were located on the tops of the sorophores, appearing like columellae of Mucorales (Fig. 3J). Such a structure has not been reported in other dictyostelids.

### Acknowledgements

I wish to thank to Dr. K. Katsumoto for kindly correcting the Latin description.

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