FORMATION OF SPORANGIA FROM CONIDIA AND HYPHAL SEGMENTS IN AN INDONESIAN BASIDIOLBUS

Charles Drechsler

CELL DIVISION in some measure suggestive of sporangial development was observed by Raciborski (1896) in Basidiobolus ranarum. Eidam (1886) when he grew the fungus in a nutrient solution that contained one per cent ammonium chloride or ammonium sulphate as nitrogen source, together with one per cent glucose and 0.025 per cent of KCl, of CaCl₂, of NaNO₂ and of MgSO₄. Growth in this solution was meager and yielded small masses of mycelium that could easily be torn apart. After two or three days, the short hyphal segments composing the mycelium became increasingly divided by longitudinal, oblique and transverse membranes. The resulting cells were set free when eventually the envelope of each parent hyphal segment broke. Thereupon they rounded up and could in their turn repeat the multiplicative development through which they had originated. Raciborski held this development similar to that shown in the pellmella stage of various algae. The same investigator reported also that when he transferred mycelium of B. ranarum to a solution containing 10 per cent glycerine, one per cent peptone and one per cent glucose, some hyphal cells grew prodigiously, often attaining a diameter of 60μ. After repeated nuclear division had taken place in the enlarged cells very thin membranes were laid down between the many resulting nuclei, though the segments delimited thereby did not separate and did not become rounded.

Levisohn (1927) placed conidia of Basidiobolus ranarum in glycerine-peptone-glucose solution of the same composition as that used by Raciborski. A large proportion of them yielded 2–8 separate globose cells in 24 hours. In several days 20–30 hyphal, rounded cells were formed. This multiplication in many instances came about entirely through repeated binary fission, with the two daughter cells derived from each division separating and becoming rounded. In many other instances, however, the conidial contents would first divide internally, forming a few endogenous cells, which, on being released when the conidial envelope had ruptured, multiplied by fission. As the globose cells, 11–16μ in diameter, formed in the glycerine solution succumbed readily to desiccation, they were not considered wholly equivalent to the "Darmform," that is, to the relatively durable spherical cells, 10–23μ in diameter, characteristic of the fungus during its sojourn within the digestive tube of the frog.

Levisohn found that when living material of Basidiobolus ranarum was fed to frogs the ingested conidia—only conidia of the globose type were mentioned by her—would begin to undergo repeated division 3–4 hr. later. Soon afterwards the conidial envelope would break apart, releasing 2–4 endogenous segments that continued to divide until in some instances 50–60 cells were formed, which after becoming rounded, commonly measured 13–16μ in diameter. These cells were found to be identical with the "Darmform." Although in respect to comparative morphology, Levisohn mentioned merely that the disintegration of the ingested conidium vividly recalled sporangial development, the ingested conidium was definitely set forth in later textbooks on mycology (Fitzpatrick, 1930; Gwynne-Vaughn and Barnes, 1937; Wolf and Wolf, 1947; Bessey, 1950) as undergoing conversion into a sporangium. Apparently no similar participation in asexual reproduction has been ascribed to hyphal segments of B. ranarum, although Levisohn found that in a frog's digestive tube such segments sometimes underwent division of their protoplasmic contents into 6–10 polyhedral or globose cells resembling the cells derived from conidia. Because ingested hyphae showed internal division less consistently and less promptly than ingested conidia, and because, further, the cells they produced varied somewhat widely in diameter (6–28μ) and contained protoplasm of granular rather than of hyaline character, Levisohn concluded that in nature the globose "Darmform" must develop exclusively from conidia.

If, as is generally believed, Basidiobolus ranarum obtains nourishment in the frog's digestive tube, the conversion of ingested conidia into the "Darmform" would seem to include sporangial development, vegetative growth and presumably some vegetative multiplication. Owing to emergence of reproductive and vegetative stages, recognition of sporangia and sporangiospores in a definitive condition might here become quite difficult. Fortunately, no troublesome confusion of developmental stages intrudes when sporangia are formed from conidia in maize-meal agar cultures of B. haptosporus Drechsler (1947), of B. meristosporus Drechsler (1955), and of the musty-smelling species widely distributed in the United States, which, more especially because of the undulated contours of its zygospores, best merits identification as B. ranarum (Drechsler, 1956). In agar cultures the conidial envelopes ordinarily remain intact until after segmentation has been completed and the delimited cells have begun to round. Conidia of the elongated adhesive type usually develop into sporangia in greater number than globose conidia. Development of sporangia in maize-meal agar cul-

---

1 Received for publication April 15, 1958.
2 Mycologist, Crop Research Division, Agricultural Research Service, United States Department of Agriculture.
tures of *B. haptopsorus* may be delayed until conidia have been produced abundantly through germination of after-ripened zygospores. Both globose and elongated sporangia can usually be found in maize-meal-agar plate cultures of *B. meristosporus* only 3 or 4 days after they were planted, but similar cultures of the odorous *B. ranarum* will usually show little or no sporangial development for 10 or 15 days after their inoculation. Among the cultures of the odorous *B. ranarum*, of *B. haptopsorus* and of *B. meristosporus* that I have isolated from frog excrement and decaying plant detritus in the United States, not any has so far been observed giving rise to sporangia in any manner other than through segmentation of globose and of elongated conidia.

Less restricted sporangial development has been seen, however, in one of the several *Basidiobolus* isolations that in a recent paper by Emmons et al. (1957) were stated to be conspecific and together were somewhat provisionally identified with *B. ranarum*. The fungus in question, isolated from a human patient in Indonesia, was kindly supplied to me by C. W. Emmons on Sabouraud's agar in a tube culture numbered A 1238. When the culture was examined 48 days after it had been planted, its slanted surface presented a somewhat folded lichenoid appearance. Numerous thick-walled chlamydospores (fig. 1) were found in the deeper portions of the folded layer, while the stout hyphae closely arranged near the surface bore equally numerous yellowish zygospores (fig. 2) containing coarsely granular protoplasm interspersed with globsules. When portions of the folded layer were transferred to Petri plates of maize-meal agar the fungus grew out into a less densely branched mycelium. A similar mycelium composed of hyphae 10–15 μ wide (fig. 3) in moderately open arrangement was developed on plates of maize-meal agar to which one per cent peptone had been added. The distal portions of elongating hyphae at the forefront of a mycelium actively expanding in a Petri plate of maize-meal agar amended by addition of one per cent peptone showed only moderate variations in width, but after the substratum was overgrown many segments gradually became distended to widths of 25–40 μ (fig. 4, 5).

Growth of the Indonesian fungus was never accompanied by the musty odor common to many species of *Streptomycetes* and given off strongly by nearly all American isolations of the *Basidiobolus* best meriting identification with *B. ranarum*.

Like *Basidiobolus meristosporus*, the Indonesian fungus usually continues throughout its period of active growth to produce phototropic propulsive aerial conidiophores from submerged and from procumbent hyphal segments. A submerged segment gives off a filamentous branch that must make its way through the overlying agar before it can ascend into the air, while a procumbent segment can directly send up a phototropic stalk with a distal swelling surmounted by a single globose conidium (fig. 6). After the conidium has been forcibly shot off, it is found in some instances still attached to an empty terminal piece of the conidiophore (fig. 7, 8). More commonly, however, the membranous piece (fig. 9–13) becomes detached while in flight and thus falls on the substratum wholly separated from the globose conidium (fig. 14–32). A conidium, whether denuded (fig. 33) of the membranous piece or not (fig. 34), may put forth a stout germ hypha capable either of developing into a new propulsive conidiophore or of branching out into a new mycelium.

Even in cultures only 2 or 3 days old, many globose conidia (fig. 35a, 65a) of the Indonesian fungus germinate by sending up a slender conidiophore on which is borne a conidium (fig. 35b, 65b) of the elongated adhesive type. Most of the adhesive conidia thus produced (fig. 36–61) closely resemble the homologous reproductive bodies of *Basidiobolus meristosporus* and the odorous *B. ranarum* in being unbranched and in having a single glandular beak. In some instances, a globose conidium (fig. 62a) gives rise to a branched adhesive conidium with plural glandular beaks (fig. 62b). Adhesive conidia display repetitive development abundantly, each (fig. 63–64a; 66–68a) putting forth a slender conidiophore on which another adhesive conidium (fig. 63–64b; 66–68b) is borne. Conidia provided with a single adhesive beak (fig. 63a, 66a) may have offspring with two adhesive beaks (fig. 63b, 66b), and, conversely, conidia with two adhesive beaks (fig. 64a, 68a) may produce single-beaked offspring (fig. 64b, 68b).

The branched conidium in many instances is rather symmetrically ypsiliform with each of its two distal lobes terminating in an adhesive beak (fig. 69–73). A less symmetrical ypsiliform shape often results when only one of the two distal lobes is provided with an adhesive beak, the other remaining unmodified at its rounded tip (fig. 74–79). Among trichotomously branched conidia, some show all three distal lobes terminating in an adhesive beak (fig. 80), while others have only two lobes (fig. 81) or only 1 lobe (fig. 82) provided with a beak. Similarly, conidia in which four distal lobes are recognizable may be provided with only two or three adhesive beaks (fig. 83), or even with only a single beak (fig. 84). On the other hand, 2–4 glandular beaks are often found crowded on the distal end of an unbranched conidium (fig. 85) or on a lobe of a branched conidium (fig. 62b). The adhesive secretion from glandular beaks only a few microns apart usually coalesces into a single mass.

Sporangia formed through segmentation of conidia have been found in maize-meal-agar plate cultures of the Indonesian fungus within four days.
after inoculation. An elongated conidium divided only by a median wall (fig. 86a, b) cannot with certainty be held to represent an early stage in the development of a sporangium, since a conidium that is to be converted into a sexual reproductive unit likewise first undergoes division into two nearly equal segments. While the first division in an elongated conidium is usually in a plane perpendicular to the longitudinal axis, the initial septum in a globose conidium may be laid down in a plane perpendicular or parallel or oblique to that axis. From the partitions formed later it is usually distinguished by its straight diametral course through the center of the sporangium (fig. 87).

Elongated conidia undergo conversion into sporangia in larger numbers than globose conidia. The sporangia developed from unbranched elongated conidia with a single adhesive beak (fig. 67b, 88–95) closely resemble the adhesive sporangia of Basidiobolus haptosporus, B. meristosporus and the odorous B. ranarum. Segmentation of a branched conidium with plural adhesive beaks yields a sporangium (fig. 96) of a type known only in the present isolation.

In Petri plate cultures of maize-meal agar containing one per cent dextrose, the Indonesian fungus produces sporangia abundantly also through conversion of hyphal segments (fig. 97a–d; 98a, b; 99a–c; 100a–c) lying close under the surface of the substrate. Numerous sporangia of such origin can be found in cultures 9 days old. Cultures 16 days old commonly show some sporangia in which the envelope has ruptured, thereby permitting gradual release of the rounded endogenous spores. In Petri plate cultures inoculated in several places especially copious development of sporangia from hyphal segments will usually occur in the terminal branching systems along the zone where the forefront of one expanding mycelium has been halted by encountering the forefront of another. Internal division of the hyphal segments presents everywhere a thoroughly normal appearance and is always accomplished without any noticeable degeneration of protoplasm. It is not accompanied by the bizarre growth effects—formation of giant cells, irregular and very pronounced thickening of the wall—that Raciborski observed when he grew Basidiobolus rana-rum in his glycercine-peptone-glucose solution. The absence of abnormality is further manifested by the wholly correct structure of mature zygospores (fig. 97e–h) produced at the same time in adjacent or neighboring portions of the same branching systems.

Sporangial development has never been observed in the conspicuously thick-walled cells, or chlamydospores, usually formed abundantly at depths more than 2 mm. below the surface of maize-meal-agar tube cultures. However, some slightly indurated segments (fig. 101), which in Petri plate cultures are found in procumbent and aerial hyphae, readily become converted into sporangia (fig. 102, 103). The enveloping wall of a sporangium having such origin usually measures about 1 μ in thickness. Many distended thin-walled segments of branches given off ascendingly from procumbent or aerial filaments likewise undergo conversion into sporangia wholly (fig. 104, 105a) or in part (fig. 105b). Instances in which a distended thin-walled segment of a shallowly submerged hyphal branch (fig. 100) or of an aerial branch (fig. 105) partly is converted into a sporangium and partly remains in a vegetative state (fig. 100d, 105c) are not anomalous, as similar development occurs also in conidia of the Indonesian fungus and of congeneric isolations (Drechsler, 1955, fig. 1 N. V).

The uninucleated spores of globose or ellipsoidal shape (fig. 106a–v) that are released when a sporangium developed from a hyphal segment gradually breaks apart resemble closely those released from sporangia of conidial origin. They...

Fig. 1–64. Basidiobolus isolation (A 1238) originally obtained from a lesion on an infected person in Indonesia; drawn as it developed on Sabouraud's agar (Fig. 1, 21), on maize-meal-peptone agar (Fig. 3–5) and on maize-meal-dextrose agar (Fig. 6–64); magnification in Fig. 1, 2, 6–64 is ×500; in Fig. 3–5 magnification is ×250.—Fig. 1. Hyphal segment containing three chlamydospores.—Fig. 2. Terminal portion of hyphal branch bearing 2 zygospores, a and b.—Fig. 3. Terminal portion of a hypha at forefront of an actively growing mycelium.—Fig. 4. Terminal portion of a branched hypha at forefront of a mycelium that has stopped growing on encountering another mycelium of the fungus.—Fig. 5. Wide segments of a mycelium similarly halted in its peripheral growth.—Fig. 6. Procumbent hyphal segment that has given rise to a phototropic conidiophore and globose conidium.—Fig. 7, 8. Globose conidia, each with attached piece of conidiophore membrane.—Fig. 9–13. Detached terminal pieces of conidiophore membranes.—Fig. 14–32. Detached globose conidia showing usual variations in size and shape.—Fig. 33. Detached globose conidium germinating.—Fig. 34. Germinating conidium to which an empty piece of conidiophore membrane is still attached.—Fig. 35. Empty globose conidium, a, from which has been extended a slender conidiophore bearing an elongated conidium, b.—Fig. 36–61. Detached unbranched elongated conidia, each with a single adhesive beak at its tip.—Fig. 62. Globose conidium, a, from which has been extended a slender conidiophore bearing a bifurcate elongated conidium, b, which is still somewhat immature as its interior is still continuous with the single glandular beak terminating one branch as well as with the 3 glandular beaks terminating the other beak.—Fig. 63. Empty unbranched elongated conidium, a, from which has been extended a slender conidiophore bearing a branched elongated conidium, b, that terminates in 2 adhesive beaks and an unmodified tip.—Fig. 64. Empty bifurcate elongated conidium, a, from which has been extended a slender conidiophore bearing an unbranched elongate conidium, b.
vary mostly between 8- and 14\(\mu\) in diameter, though specimens 15-20\(\mu\) in diameter (fig. 107a, b) can usually be seen in some number. These larger specimens can, in many instances, undergo further division (fig. 108-110) and thus are transformed into 2-6 smaller spores. Apparently cell division here is not preceded or accompanied by vegetative growth. The belated divisions presumably take place only where division within the sporangium did not proceed to an ultimate stage. They would seem analogous to the supplementary divisions observable in the production of plural zoospores from oversized cysts resulting from occasionally incomplete cleavage of protoplasm within sporangia of various water molds, including, for example, my Aphanomyces cochlioides (Drechsler, 1929, p. 314, fig. 1E).

Sporules of the Indonesian Basidiobolus that are developed in sporangia of conidial origin usually contain protoplasm of nearly homogeneous appearance, whereas those formed in sporangia originating through internal division of submerged hyphal segments are commonly filled with noticeably granular protoplasm. However, the sporangia resulting from conversion of somewhat indurated segments of aerial hyphal branches generally yield spores in which the protoplasm surrounding the single nucleus appears to be virtually homogeneous. The texture of its protoplasmic contents would seem, therefore, to indicate less directly whether a newly released spore came from a sporangium of mycelial or conidial origin than whether it was formed within a submerged or an aerial sporangium.

In the Indonesian fungus, as in Basidiobolus meristosporus, sexual reproduction takes place concurrently with asexual reproduction and yields globose smooth-walled zygospores (fig. 2a, b; 97e-h; 111). The smooth zygospores are in disagreement with the morphology of B. ranarum, for according to Eidam's (1886, p. 221, lines 2-3) original account, the zygospore of that species acquires undulated contours — "die bisher aussen glatte Spore bekommt wellige Umrisse" — during the later stages of development. Fairchild (1896) figured mature zygospores of B. ranarum as having an undulated wall-layer, and Levisohn (1927) characterized them as being mostly polyhedral, but, on the whole, European writers have given little comment bearing on zygospore sculpture as a diagnostic feature of the species. Although the one figure in which Eidam illustrated sexual reproductive apparatus of his B. lacertae shows the zygospore as being surrounded by a smooth wall, this author did not mention smooth contours of its zygospores among the several features by which he held B. lacertae distinguished from B. ranarum. Since the one zygospore was figured and described as being orange-yellow and as showing a "heller Fleck" in its center, it had presumably attained a fully mature condition. Nevertheless, the obviously smooth zygospore wall in Eidam's illustration was ignored also by Levisohn when she argued that B. lacertae was identical with B. ranarum. At all

Fig. 65-111. Basidiobolus isolation (A 1238) originally obtained from a lesion on an infected person in Indonesia; drawn as it developed on Petri plates of maize-meal agar containing 1 per cent dextrose; \(\times 500\) throughout. - Fig. 65. Empty globose conidium, a, from which has been extended a slender conidiophore bearing an unbranched elongated conidium, b. - Fig. 66. Empty elongated conidium, a, from which has been extended a short slender conidiophore bearing a bifurcate elongated conidium, b. - Fig. 67. Empty unbranched elongated conidium, a, from which has been extended a slender conidiophore that supports an elongated unbranched sporangium, b. - Fig. 68. Empty elongated conidium, a, provided with 2 adhesive beaks and a slender germ conidiophore bearing an unbranched elongated conidium, b, which, being still continuous with its glanular beak, is somewhat immature. - Fig. 69-73. Detached bifurcate elongated conidia, each with 2 adhesive beaks. - Fig. 74-79. Detached bifurcate elongated conidia, each having one unmodified distal lobe in addition to the lobe bearing an adhesive beak. - Fig. 80. Trichotomously branched elongated conidium with 3 adhesive beaks. - Fig. 81. Trichotomously branched elongated conidium with 1 adhesive beak. - Fig. 82. Trichotomously branched elongated conidium with 1 adhesive beak. - Fig. 83. Tetrachotomous elongated conidium provided with 2 adhesive beaks. - Fig. 84. Tetrachotomous elongated conidium with 1 adhesive beak. - Fig. 85. Elongated conidium with 4 glanular beaks on its wide distal end. - Fig. 86. Two elongated conidia, a and b, that have each become divided by a median cross-wall. - Fig. 87. Globose sporangium formed through internal segmentation of a globose conidium to which an empty piece of conidiophore membrane remained attached. - Fig. 88-95. Unbranched elongated sporangia, each with a single adhesive beak. - Fig. 96. Trichotomous elongated sporangium with 2 adhesive beaks. - Fig. 97. Shallowly submerged branched hypha at periphery of a mycelium in a culture 16 days old; a, d, sporangia; e-h, mature zygospores. - Fig. 98. Hyphal branch with 2 sporangia, a and b, present in a culture 9 days old. - Fig. 99. Hyphal branch with 3 sporangia, a-c, found in a culture 11 days old. - Fig. 100. Hyphal branch with 3 sporangia, a-c, and a vegetative cell, d; found in a culture 9 days old. - Fig. 101. Thick-walled segment of an aerial hypha present in a culture 14 days old. - Fig. 102, 103. Sporangia developed from thick-walled segments of aerial hyphae present in a culture 14 days old. - Fig. 104. Wide aerial hyphal branch in which a sporangium has been formed; found in a culture 14 days old. - Fig. 105. Wide aerial hyphal branch containing 2 sporangia, a and b, as well as a vegetative cell, c; found in a culture 11 days old. - Fig. 106. Sporangiospores, a-e, released from sporangia of hyphal origin, showing usual variations in size and shape. - Fig. 107. Larger sporangiospores, a and b, likewise from sporangia of hyphal origin, and possibly still capable of further division. - Fig. 108-110. Globose bodies composed of several young sporangiospores which had not separated when released from sporangium. - Fig. 111. Mature sexual reproductive unit that is unusual in showing 2 septa in each of the opposed beak-like protuberances.
events, the Indonesian fungus departs from the morphology ascribed to *B. lucerinae* in that the paired beak-like protuberances on its mature sexual reproductive units are not short and asetose but are well developed and contain one or occasionally two cross-walls.

Until an ample collection of *Basidiobolus* cultures from tropical or subtropical lands has been studied, it may remain uncertain whether the formation of branched elongated conidia in the Indonesian fungus and the production of sporangia from hyphal segments are to be regarded as attributes of a separate species or, perhaps, as expressions of developmental variation in *B. meristosporus*. The development of sporangiospores here without intervention of large conidia recalls the asexual reproduction of *Meristacrum asterospermum* Drechsler (1940) and *Ballocephala sphaerospora* Drechsler (1951) in which the hyphal segment puts forth a filament that gives rise distally to many small exogenous spores presumably homologous with sporangiospores.

**Summary**

Both globose and elongated conidia are produced in an isolation of *Basidiobolus* which after having been isolated from a human patient in Indonesia was provisionally identified with *B. ranarum*. Some elongated conidia are variously branched and thus terminate in 2–4 recognizable lobes. Not all lobes are provided with an adhesive beak, though some lobes as well as some unbranched conidia bear 2–4 glandular protuberances. Some globose and many elongated conidia become converted into sporangia through internal segmentation. In plate cultures of maize-meal agar containing one per cent dextrose, sporangia are abundantly produced also through internal segmentation of shallowly submerged hyphal segments. Sexual reproduction proceeds concurrently with asexual reproduction and yields smooth zygospores rather than zygospores of undulate contour.

**Plant Industry Station**

Beltsville, Maryland

---

**Literature Cited**


———. 1940. Three fungi destructive to free-living terri-


———. 1947. A *Basidiobolus* producing elongated second-


———. 1956. Supplementary developmental stages of

*Basidiobolus ranarum* and *Basidiobolus haptoporus*. Mycologia 48: 655–676.


Rachorski, M. 1896. Ueber den Einfluss äusserer Be-
