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Studies on Species of Conidiobolus from India-II.

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With plates II—III.

A species of Conidiobolus characterised by large conidia was frequently isolated from leaf mold collected in Poona. On maize-meal agar and sorghum meal casein decoction agar exposed to the moist detritus, numerous colonies of the fungus developed after 48—72 hours in the form of tiny inconspicuous groups of mycelium. When fresh media were exposed to the detritus, the same fungus appeared during the subsequent 7 to 10 days indicating an abundant and prolonged conidial discharge. Individual colonies were subcultured on yeast glucose agar slants and the morphological characters of the fungus studied.

The fungus makes good growth on this medium and the colony expands by the radial elongation of the vegetative hyphae as well as by the formation of daughter colonies developing from germinating conidia. The vegetative hyphae are 8—15 μ in diameter with dense granular cytoplasm. The mycelium becomes septate at an early stage (Fig. 1) and following septation becomes disjointed into segments usually 600—800 μ long alternating with empty portions of the hyphae. Often the empty wall collapses resulting in the hyphal segments becoming separated.

The conidiophores arise as unbranched phototrophic laterals of the basal mycelium extending 30—200 μ (commonly 60—80 μ) into the air and developing terminally a large globose conidium (Fig. 2). When a conidium attains the full size it is delimited by a well developed septum from the conidiophore. It is forcibly discharged to a distance of several millimeters by the sudden eversion of the separating membrane. The conidia are globose or pyriform due to the large conical or paraboloid papilla at the base, 30—50 μ in diameter with the papilla measuring 8—15 μ wide and 6—12 μ high. Often a vacuole is included above the papilla and the numerous granules present in the conidium are uniformly distributed or show a tendency towards concentration in a central area surrounded by a zone of homogenous cytoplasm (Fig. 3). When numerous conidia are discharged simultaneously. they form within 24 hours of transfer a visible cloud on the glass above the medium.

The conidia falling on the nutrient medium germinate immediately by putting forth one or more (upto 4) germ tubes (Fig. 5) which rapidly elongate to form the vegetative mycelium. Alternately a primary conidium effects a transfer of its contents through a secondary coni-

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diophore upto 50 μ long and 14 μ wide and organises a globose secondary conidium at the apex of the conidiophore. When fully formed the secondary conidium is separated by a membrane from the conidiophore (Fig. 4) and is forcibly discharged. The secondary conidia, which are smaller in size compared to the primary conidia, also show repetitional development forming globose conidia of smaller size. As a result, wide variation in conidial size (15—50 μ) is observed even in young cultures.

Several conidia also give rise to microconidia on radial sterigmata. This is more commonly observed in the conidia forming a dense cloud on the glass surface than in conidia falling on the agar medium. In forming the microconidia, the conidium develops 3—20 (most commonly 6—12) protuberances which elongate and develop into sterigmata 5—16 μ long and 2,5—5 μ broad. Each sterigma bears a globose or ovate-ellipsoid microconidium, 12—21 × 10—14 μ with a tiny papilla 2—3 μ high and 2,5—4 μ wide (Fig. 6). The microconidia are discharged and germinate immediately by developing a germ-tube (Fig. 7), which elongates to form a mycelium usually 3—7 μ wide. The wall of the primary conidium collapses following the liberation of microconidia and the sterigmata sometimes remain persistent.

When inoculated into sterilized egg yolk, the fungus makes very rapid growth consuming the medium completely. The colony shows a convoluted surface and numerous conidia are formed. Several conidia formed also show development of microconidia.

The fungus was also grown under submerged conditions on a rotary shaker. When inoculated in yeast glucose medium in 500 c. c. Erlenmeyer flasks and grown under aerated-agitated conditions rapid growth in the form of thick masses of mycelium is observed within 2—3 days. The mycelium often shows segmentation and numerous fully developed conidia are observed when the growth formed under submerged coniditions is examined.

Regarding the identity of the fungus, a comparison has to be made with species of Conidiobolus reported to show development of microconidia. It differs from C. brefeldianus (Couch, 1939) in the absence of zygospores and does not form chlamydospores as reported in *C. chlamydosporus* (Drechsler 1955). It is closely related to *C. megalotocus* (Drechsler, 1956) which has been described from the United States, but differs from it in its unbranched conidiophore, number of microconidia formed, and some minor variations in spore size. The fungus under study is referred to the same species but presented as a new variety.

Conidiobolus megalotocus Drechsl. var. **indicus** var. nov.

Mycelium colourless, branched, vegetative hyphae 8—15 μ wide (those originating from microconidia only 2—7 μ wide), becoming

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disjointed and later separated into individual segments 600—800 μ or more in length; conidiophores arising from basal mycelium, unbranched, phototrophic, extending 30—200 μ in the air (commonly 60—80 μ) and developing globose conidia terminally. Conidia forcibly discharged and accumulating as dense cloud above the culture, globose or pyriform with granular contents, the granules uniformly distributed or concentrated in a central region of conidia; primary conidia 30—50 μ in diameter with a large conical or paraboloid basal papilla 8—15 μ wide and 6—12 μ high. Secondary conidia formed by repetitional development, globose, 15—40 μ in diameter. Microconidia 3—20 (commonly 6—12) formed on radial sterigmata 5—16 × 2,5—5 μ; conidia globose or ovate-ellipsoidal 12—21 × 10—14 μ with a tiny basal papilla 2—3 × 2,5—4 μ.

Isolated from leaf mold, Poona, 10—5—1961.

A typo differt conidiophoris simplicibus nec ramosis, conidiis primariis 30—50 μ diam., papillula basali conica vel paraboloidea, 8—15 μ lata, 6—12 μ longa praedita. Conidia secundaria globosa 15—40 μ diam. Microconidia 3—20, plerumque 6—12 in sterigmatibus radiantibus 5—16/2,5—5 μ orta, globosa vel ovoideo-ellipsoidea, 12—21/10—14 μ, papillula basali 2—3/2,5—4 μ praedita.

HACC-125 (Type) deposited in Commonwealth Mycological Institute. Kew England. American Type Culture Collection, U.S.A., Centraalbureau Voor Schimmelcultures Holland and Indian Agriculture Research Institute, New Delhi.

An interesting species of *Conidiobolus* showing numerous zygospores was isolated from leaf mold collected in Poona during rainy season in June 1961. On maizemeal agar, the colonies are inconspicuous appearing as a diffuse fuzz. Under the microscope, the characteristic mycelial segments and conidia of *Conidiobolus* can be seen. Individual colonies were sub-cultured on yeast glucose agar on which after 3 days incubation dull brown colonies 3,5—4 cms. in diameter are evident. When exposed to unilateral illumination, most of the conidia are discharged in the direction of light showing a phototrophic response.

The mycelium at first is coenocytic with numerous granules, thin walled, 5—8 μ wide. Later the hyphae become septate (fig. 8) and get separated into fragments measuring 40—70 μ. The segments often separate out and become free of the main hyphae. A distinguishing character of this species is the early formation of zygospores. Usually the adjacent hyphal segments belonging to the same parent hypha fuse and conjugation sometimes takes place also between segments from different hyphae. Initially the fusing segments are of the same size but at the time of conjugation one of the segments enlarges appreciably resulting in an anisogamous condition (fig. 10). The smaller gametangium effects a transfer of its contents into the larger one, and the zygospore develops in the latter. The product of fusion appears

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as a globose body with a distinct 2-layered wall and several globose refractive globules (fig. 11). Mature zygospores are smooth, globose or subglobose with slightly protruded apex, 18—30 μ in diameter with a wall 1,5—4 μ thick. An excentric refractive globule 14—20 μ in diameter is present in the mature zygospores (fig. 12).

The conidiophores are developed in young cultures as numerous, phototrophic branches of the hyphal segments and are 15—30 μ long. A globose conidium is borne at the end of each conidiophore and is forcibly discharged at maturity. These can be observed in agar slant cultures on the top surface of the tube. Mature conidia are globose with granular cytoplasm. 20—30 μ wide and 23—36 μ long including a distinctly conical basal papilla 4—7 μ high and 5—9 μ wide (Fig. 9). They readily germinate forming one or two germ tubes.

Colony on sterilized egg-yolk medium is greyish white, covering the medium completely in 3—4 days. Conidia and zygospores are produced in large numbers on this medium.

When inoculated in yeast glucose medium and incubated on rotary shaker at 28° C, the fungus develops several small pellets during the first 2—3 days. Later, the pellets gelatinise to give rise to small hyphal segments. Conidia and zygospores are often observed when the culture is examined.

Comparative studies with species of *Conidiobolus* described so far indicates that it comes nearest to *C. brefeldianus* described by Couch (1939). The points of similarity are as follows.:

	C. brefeldianus	*Conidiobolus sp. from Poona.*
I) Mycelium	Hyphae 5,4—8,4 μ, branched, segments 50 to several hundred microns long (usually 300—420 μ).	Hyphae 5—8 μ, branched, segments 40—70 μ long and 6—9 μ wide.
II) Conidiophore	Unbranched, phototrophic, 30—50 μ × 7—10 μ.	Unbranched, phototrophic 15—30 μ × 6—9 μ.
III) Conidia	Spherical, 10—31 μ (usually 20 μ) with apiculus 7,5 μ wide and 5—7,5 μ long.	Spherical, 20—30 μ wide and 23—36 μ long. Basal papilla 5—9 μ wide and 4—7 μ high.
IV) Zygospores	Formed by fusion between anisogamous gametangia, smooth, 18—33 μ in diameter (usually 23 μ).	Formed by fusion between anisogamous gametangia, smooth, 18—30 μ in diameter with wall 1,5—4 μ thick and globule 14—20 μ in diameter.

The above data indicates that the fungus under study isolated from leaf mold in Poona is identical with *C. brefeldianus* Couch., which is a new record for India.

An interesting species of *Conidiobolus* characterised by rapid

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mycelial growth was found associated with *Empusa muscae* Cohn on dead flies. The fungus was isolated in pure culture for further studies. On maizemeal agar plates the colony rapidly expands, attaining a diameter of 20—40 mms. within 48 hours and appearing as whitish translucent growth. When sub-cultured on yeast glucose agar the radial diameter was 18 to 20 mms. within 24 hours. The colony is smooth, flat and dull white in colour. The young hyphae are coenocytic, thin-walled, 7—10 μ wide and with homogenous contents. The tips of these hyphae are broadly obtuse. Short lateral branches are formed in older hyphae, which show granular cytoplasm and vacuolation. Ultimately due to septation, mycelium gets separated into hyphal segments interconnected by hyphal membrane.

Conidiophores develop after prolonged vegetative phase, first developing as tiny whitish masses in scattered concentric bands. The hyphal segments intertwine to form a basal hyphal knot from which the conidiophores arise as short aerial branches extending 12—40 μ and developing conidia terminally (Fig. 13). Many of the hyphal knots give rise to tubular aerial branches which extend several microns in the air (Fig. 14). Conidiophores are sometimes seen to arise directly from the basal mycelium. The conidia when mature are delimited from the conidiophore by a septum. Mature conidia are globose to pyriform 22—26 μ wide and 25—33 μ long with a blunt basal papilla 2—4 μ high and 4—7 μ wide (Fig. 15). Very few conidia show repetitional development, while most of them germinate directly by one or two germ tubes.

In cultures more than 6—7 days old, zygospore formation is initiated through hyphal fusions established between hyphal segments. Following the development and maturation of the zygospores, the smooth surface of the colony is thrown into deep folds. Zygospores are globose, subglobose to ovate, smooth 23—32 μ in diameter with a wall 1—2 μ thick and a refractive globule 14—18 μ in diameter (Fig. 16).

When inoculated into egg yolk medium, a very rapid growth resulting in a deeply lobate grayish brown colony is observed. The hyphae are composed of short segments 50—100 × 8—10 μ. In older cultures zygospores are formed.

Under submerged conditions of growth on a rotary shaker in yeast-glucose medium, several large pellets are produced within three days, which on continued incubation gelatinise to give rise to hyphal segments. No conidia or zygospores were observed in cultures upto 7 days.

The fungus under-study shows certain distinctive features such as the formation of hyphal knots from which conidiophores and tubular aerial branches are formed. This is a characteristic feature not encountered in any of the other *Conidiobolus* species.

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Conidiobolus stromoideus sp. nov.

Colony on yeast glucose medium smooth, flat, extending upto 2 cms. in 24 hours; mycelium colourless, thin-walled, coenocytic with broadly obtuse apex, 7—9 μ wide, becoming septate to form hyphal segments 45—200 μ remaining inter-connected by the hyphal membrane or free; conidiophores arising as upward branches from hyphal knots formed by the mycelium irregularly interwoven, 12—40 μ high and bearing conidia terminally; conidia globose to pyriform 22—26 μ wide and 25—33 μ long, basal papilla 2—4 μ high and 4—7 μ wide; zygospores globose, subglobose to ovate, smooth, 23—32 μ in diameter with wall 1—2 μ thick and refractive globule 14—18 μ in diameter.

Associated with dead flies infected by *Empusa muscae* Cohn; July 1961. HACC-126 (Type) deposited in Commonwealth Mycological Institute, Kew, England, American Type Culture Collection, U.S.A., Centraalbureau Voor Schimmelcultures, Holland and Indian Agriculture Research Institute, New Delhi.

Mycelium hyalinum, tenuiter tunicatum coenocyticum, antice late obtusum, 7—9 μ latum, postea in segmenta 45—200 μ longa, nunc membrana hyphali connexa, nunc libera divisa; conidiophora e nodulis hypharum orta, recta, 12—40 μ alta; conidia acrogena, globosa vel piriformia, 22—26 lata, 25—30 μ longa, papillula basali 4—7 μ lata, 2—4 μ longa praedita; zygosporae globosae, subglobosae vel ovoideae, leves, 23—32 μ diam., episporo 1—2 crasso, guttula oleosa, 14—16 μ diam. praedita.

Summary.

Morphological and cultural studies on three species of *Conidiobolus* isolated from Poona are presented. The first species forming large conidia, which develop 3—20 microconidia on radial sterigmata is presented as a new variety of *Conidiobolus megalotocus* Drechsl. under the name. *C. megalotocus* var. *indicus*. A second species, identical with *C. brefeldianus* Couch forms numerous zygospores through anisogamous conjugation between hyphal segments. *C. stromoideus* is described as a new species characterised by the formation of conidiophores and tubular aerial branches arising from basal hyphal knots. Numerous smooth-walled zygospores are also formed by this species.

Grateful thanks are due to Professor Dr. Franz Petrak for kindly giving the latin diagnosis of the new species.

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Explanation of Plate II—III.

Figs. 1—7. *Conidiobolus megalotocus* var. *indicus*. — 1. Young assimilative hypha × 700. — 2. Conidiophore showing developing conidium ×800. — 3. Conidium × 1000. — 4. Formation of secondary conidium × 1000. — 5. Germination of conidium by germ tube × 1000. — 6. Formation of microconidia on radial sterigmata ×1000. — 7. Germination of microconidium × 1500.

Figs. 8—12. *Conidiobolus brefeldianus*. — 8. Vegetative mycelium × 1000. — 9. Conidium × 1000. — 10. Anisogamous gametangia × 1000. — 11. Young zygospore × 1000. — 12. Mature zygospore × 1000.

Figs. 13—16. *Conidiobolus stromoideus*. — 13. Hyphal knot developing conidiophore × 250. — 14. Hyphal knot developing tubular aerial branches × 250. — 15. Conidium × 1000 —. 16. Zygospore × 1000.

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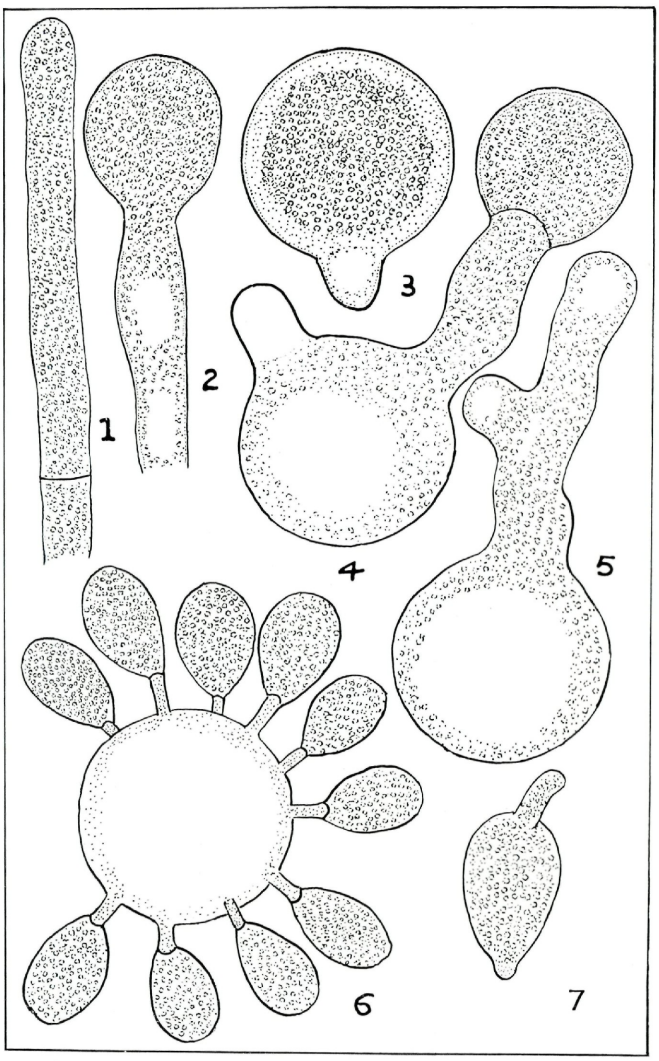

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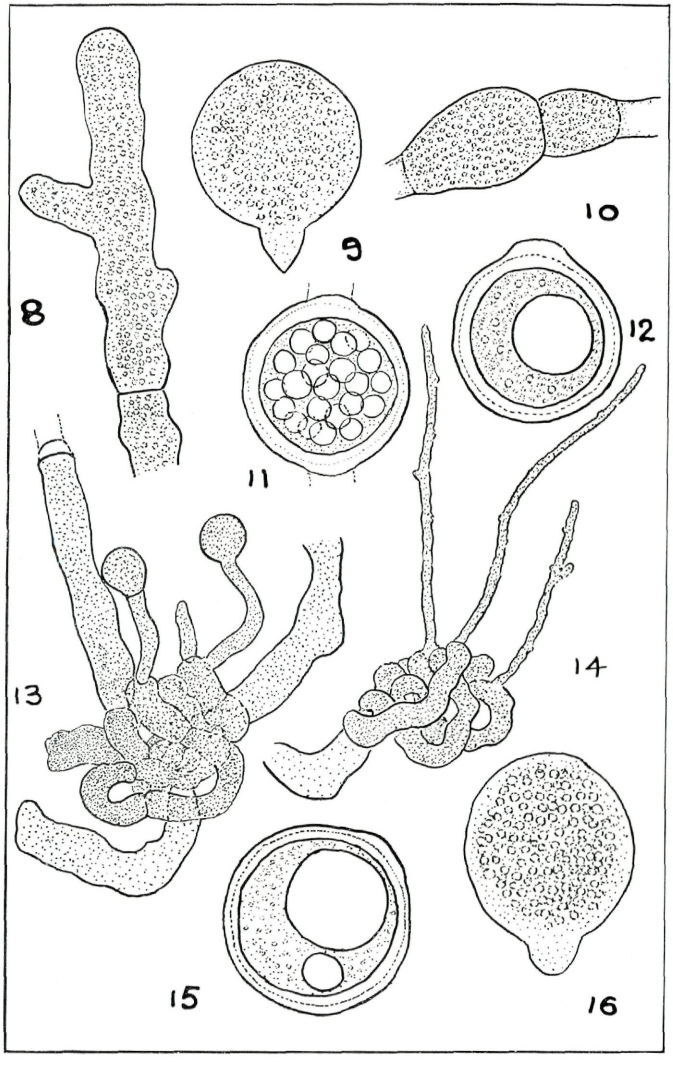

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Zeitschrift/Journal: Sydowia

Jahr/Year: 1962/1963

Band/Volume: 16

Autor(en)/Author(s): Srinivasan M. C., Thirumalachar M. J.

Artikel/Article: Studies on Species of Conidiobolus from India-II. 60-66