TWO NEW FAMILIES OF MUCORALES

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SUMMARY

Two new families of Mucorales are described. Saksenaeaceae Hesseltine & Ellis is typified by Saksenaea Saksena and includes Echinosporangium Malloch. Radiomycetaceae Hesseltine & Ellis is typified by Radiomyces Embree and includes Hesseltinella Upadhyay. Characteristics of the genera are summarized.

For some time it has been apparent that the genera Saksenaea Saksena, Echinosporangium Malloch, Radiomyces Embree, and Hesseltinella Upadhyay do not fit well into the known families of Mucorales. Consequently, two new families centered around Saksenaea and Radiomyces are described here.

Saksenaeaceae Hesseltine & Ellis, fam. nov.

Hyphae floccosae, aeriae, plerumque aseptatae; hyphae substrati large ramosae; sporangia fiunt aeria, ampulliformia seu butuliformia, numquam globosa seu pyriformis; sporangia aut habentia aut carentia columellae; mm sporangiorum politi, seu paucas habentes spinas, persistentes; sporangiola conidiaque non formantar. Zygospori ignoti.

Aerial hyphae floccose, generally aseptate; substrate hyphae abundantly branched; sporangia borne aerially, flask shaped or sausage shaped, never globose or pyriform; sporangia with or without columellae; sporangial wall smooth or with a few spines, persistent; sporangiola and conidia not formed. Zygospores unknown.

Type genus: Saksenaea Saksena, Mycologia 45: 434. 1953.

This family contains two monotypic genera Saksenaea (Saksena, 1953) and Echinosporangium (Malloch, 1967).

Saksena (1953) has given us the only detailed account of the morphology of Saksenaea vasiformis, the type and only known species of the genus. He discovered it twice in forest soil from the Madhya Pradesh State near Sagar, India, and named it for R. K. Saksena, his major professor.
In pure culture *S. vasiformis* (Fig. 1), grows rapidly, producing profuse flocculant aerial hyphae with little sporulation and a large amount of many-branched submerged hyphae. At first the hyphae are aseptate, but later a few septa develop. Since sporulation invariably tends to diminish in pure culture, sterile cultures often result. Saksena reported that if pieces of potato dextrose agar permeated with mycelium are floated on sterile distilled water, sporangia form abundantly. We have obtained sparse sporulation on Czapek's solution agar or on hay extract agar.

Sporulation occurs only in the following fashion: Sporangia are formed at the extremities of short unbranched hyphae when the fungus is grown on water. At this point a strongly dichotomously branched rhizoidal system is formed. In agar the dichotomy is not so apparent. Above and opposite the rhizoids, a small protuberance is put forth and develops into a peculiar sporangium. The sporangium is separated from the rhizoids by a short hypha. At the same time the rhizoids and sporangium are cut off by a septum. The rhizoidal branches in water are blunt and show radial symmetry; they are somewhat tapered in agar.

The sporangial primordium elongates, is larger in diameter than the vegetative hyphae, and is pointed at its apex. Later, a portion above the hypha attached to the rhizoids swells into a spherical venterlike struc-
tured, and the upper portion of the young sporangium elongates and forms a long neck, the end of which swells slightly. Hence, the sporangium gives the appearance of a stalked round-bottomed flask. The dense cytoplasm in the venter and neck now becomes differentiated into sporangiospores that fill the venter and most of the neck of the sporangium. At the same time that sporangiospores are being formed by cleavage, a dome-shaped columella is formed in the bottom of the venter. The mature sporangium has a thin, hyaline, smooth wall while the rhizoids and the stalk of hyphae bearing the sporangium become thick walled and brown. Typically only one sporangium is formed above the rhizoids; occasionally a second sporangium may be formed simultaneously or successively.

The sporangiospores at maturity fill the entire sporangial cavity. Finally the tip of the neck of the sporangium dissolves and the small, hyaline, oblong spores extrude. The spores are nonmotile and tend to accumulate at the tip of the neck. Sporangiospores germinate by forming short tubes, as is seen in other Mucorales. No zygospores have ever been observed.

The evidence that Saksenaea belongs to the Mucorales is based on the following facts regarding S. vasiformis:
1. The hyphae are nonseptate.
2. Aerial sporangia are formed that produce aplanospores.
3. Columellae are formed as in the Mucorales.

Saksena believed that Saksena was related to the Mucoraceae because in S. vasiformis no other secondary spores or conidia were formed, but on the other hand, the dichotomous rhizoids suggested a relationship to Syncephalis spp. Neither the flask-shaped sporangia nor the release of spores when the apex of the sporangial neck is dissolved represents conditions found in any other member of the Mucorales. Saksena also noted that the release of spores from the sporangium recalls the condition in species of Catenomyces. Also the shape of the sporangium reminds one of the condition in Nephrochytrium and Catenomyces, but representatives of these genera have motile zoospores. When the zygospores are discovered, a more exact position of Saksenaea can be determined.

The only physiological studies of S. vasiformis are reported in the papers by Baijal (1967) and Tiwari (1955). Tiwari studied media for sporulation and concluded that Czapek’s medium was best for growth and that a soil extract medium of unknown composition was best for sporulation. No synthetic media would support sporulation. Baijal (1967) showed that the fungus would grow between 15 and 39 C with an optimum range of from 30 to 35 C. The best pH was about 6.0, but
growth could occur from $pH$ 2.5 to 11.5. The fungus could use a wide range of carbon sources with the best, as measured by dry weight, being fructose, glucose, mannose, sucrose, starch, mannitol, and sorbitol. However, sporulation was highest on certain carbon compounds which did not give high dry weights; namely, arabinose, rhamnose, and lactose. Nitrogen sources giving the best dry weights with glucose as the carbohydrate source were ammonium nitrate, ammonium chloride, valine, aspartic acid, glutamic acid, asparagine, and peptone.

Saksenaea was placed in the Mucoraceae by Hesseltine (1955). Boedijn (1958) thought it ought to go into a separate family. Zycha and Siepmann (1969) also placed it in the Mucoraceae but placed Echinosporangium in the Mortierellaceae without any discussion of their reasons.

Since its initial isolation from soil in the Patharia Forest in India in 1953, S. vasiformis has been isolated from widespread tropical sources and appears to be worldwide in distribution. Farrow (1954) found it in mixed clay and humus soil of Barro Colorado Island. In 1962, Hodges recorded it from southern forest-tree nursery soil in Georgia as the first record in the United States. It was isolated from immersion tubes in Honduras soil from banana plantations by Goos (1963). Joffe and Borut (1966) reported it once from groundnut soil of Israel. It has also been isolated from heavily manured soil of Allahabad (Baijal, 1967). Recently two different isolates were sent to us from burn patients.

Echinosporangium also contains only one species E. transversalis Malloch (Fig. 2), and likewise, its zygosporic state remains undiscovered. Echinosporangium transversalis was isolated from soil in Nevada by Malloch (1967), from soil of the Sonora desert in Mexico by Ranzoni (1968), and from Texas soil by C. J. Alexopoulos.\footnote{A culture listed in the Tenth Edition of the American Type Culture Collection Catalog, 1972.}

The information on E. transversalis is even less complete than on S. vasiformis. The only morphological study on the fungus is the paper by Malloch (1967) who isolated it from soils with a $pH$ of 6.5 that were collected near Virginia City, Nevada. The fungus grows readily on most standard media used for growth of fungi. Sporangiophores are formed on aerial mycelium which, in places, branches dichotomously until usually eight branches are formed. The apex of each of these branches again divides dichotomously and becomes differentiated into sporangia. At first the young sporangia are septate, but later these septa disappear. Just before sporangiospore differentiation, spines develop at the apices.
At the base of each spine are two pores. The spores are differentiated from the apices to the center of the sporangium and often there is a piece of undifferentiated protoplasm in the sporangium which superficially suggests a columella. The undifferentiated protoplasm is completely surrounded by a membrane and hence not attached to the sporangiophore as are true columellae.

Sporangiospores of *E. transversalis* are hyaline, smooth, irregular in size and shape, and nonmotile. They germinate by one or more germ tubes and, unlike *S. vasiformis*, are not extruded from the sporangium. The sporangia are unlike anything else seen in the Mucorales; they are transversely elongate, cylindrical, or sausage-shaped with a few spines at their apices. Malloch believed *E. transversalis* was a member of the Mucorales because of its nonseptate, hyaline, aerial sporangia and its nonmotile spores. The lack of a true columella and its peculiarly shaped sporangia would exclude it from the Mucoraceae. It is not a member of the Mortierellaceae because its sporangia are never globose.

This new family is admittedly an unnatural one since zygospores are unknown in the single known species of *Saksenaea* and *Echinosporangium*. For this reason we are not certain that these genera are closely related. Their sporangia are unlike any other in the Mucorales. Those of *E. transversalis* are transversely elongate, cylindrical, with one to five spines at each end and are formed on dichotomously branched sporangiophores. The sporangia of *S. vasiformis* are stalked and flask shaped with a long neck. A distinct columella is delimited at the base of the venter. Typically the sporangia of *S. vasiformis* form singly at the end of a sporangiophore that arises from a hypha a short distance above the rhizoids.

Putting *Saksenaea* and *Echinosporangium* together in a new family can be justified because neither can possibly be placed in the recognized families of Mucorales as they currently exist. The characteristics of their type species which place them together are: (1) Both have sporangia that are neither globose nor pyriform; (2) no secondary type of asexual reproduction is known; (3) either a dichotomously divided sporangiophore or rhizoid-like mycelium is produced at or near the base of the sporangium; (4) each grows rapidly and produces hyaline mycelium; (5) the sporangia are borne on aerial hyphae; (6) each has multisporous sporangia; and finally (7) they are predominately soil-inhabiting forms.

Radiomycetaceae Hesseltine & Ellis, fam. nov.

Sporangiophori orti ex hyphis aeriiis seu stolonibus; adsunt rhizoideae; sporangiophori primarii terminantur vesiculis, a quibus oriuntur radiate vesiculi
Sporangiophores arising from aerial hyphae or from stolons; rhizoids present; primary sporangiophore terminating in a vesicle from which secondary stalked vesicles arise radiately: unisporous or multisporous sporangioles short stalked or nearly sessile, arising from secondary vesicles; columellate sporangia absent; zygospores where known smooth walled, light brown, with appendiculate suspensors; homothallic or heterothallic.


This family contains the two genera Radiomyces (Embree, 1959) and Hesseltinella (Upadhyay, 1970). Both genera were originally placed in the Thamnidiaceae by their discoverers, but Zycha and Siepmann (1969) placed Radiomyces in the Choanephoraceae without an explanation.

The genus Radiomyces contains two species. Radiomyces spectabilis Embree (1959), the type species, was found on lizard dung collected in Mono County, California. Radiomyces embreei Benjamin (1960) was found on mouse dung in San Bernardino County, California, and from soil of California and Arizona in the Sonoran desert by Ranzoni (1968).

Embree made a detailed study and illustrated R. spectabilis (Figs. 3–4) from both the natural substrate and in pure culture on Y PSs agar (Difco 2). Sporangiospores germinate rapidly on this medium and produce 1–3 germ tubes which develop substrate mycelium. Within 2–3 days both asexual and sexual reproductive structures begin to form. Stolons which produce many-branched rhizoids develop, and sporangiophores arise on the stolons a short distance from the rhizoids. One or two sporangiophores are formed, and these develop a terminal primary vesicle. From this primary vesicle secondary uniseptate sporangiophore stalks develop which form secondary vesicles at their tips. From the surface of the secondary vesicles arise tertiary stalks each of which develops a single sporangium at its tip. The sporangioles are covered with long, terminally knobbed papilliae, and the sporangiospores are released when the wall bursts in water.

Sexual reproduction occurs by the fusion of gametangia as in Mucor. After fusion, a whorl of projections appears on both suspensors, and

2 The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.
these branched projections then surround the zygospore. The projections become thick walled and brown. The mature zygospore is smooth walled except for two circular scars at the attachment of the suspensors. The zygospore wall consists of two layers that can be readily separated from each other. At first the contents consist of numerous small oil droplets which later coalesce and form a single large one.

Embree noted that the characteristics of the genus were like the Mucoraceae in some respects and like those of the Thamnidiaceae in others (the sporangioles). According to precedent in the Mucorales, the genus was placed in the Thamnidiaceae, but Embree suggested that a new family might have to be established for it.

The second species of Radiomyces, R. embreei R. K. Benjamin, is similar to the type species but differs in having vesicles which are long clavate; septa are found just below the fertile regions, and the ovoid sporangioles contain single sporangiospores. The sporangiolar wall has small knobbed appendages.

Hesseltinella is represented by the type, H. vesiculosa Upadhyay (1970) (Figs. 5-6), found by its author in paddy fields in the states of Maranhão and Pernambuco in Brazil. This species grows and sporulates on a variety of cultural media and has optimum growth at 28°C with a temperature range of from 15 to 37°C.

The sporangiophores arise from the substrate mycelium or from stolons and at their ends develop a terminal swelling with a short sterile protuberance above. From the vesicle, secondary branches develop in a radiate fashion and each of these forms an apical secondary swelling that bears terminally a single sporangiole on a short stalk. Later the sporangiophore may form a small number of lateral and intercalary swellings. From these swellings secondary branches radiate which may be simple or branched and terminate in sporangioles. The fungus produces stolons and poorly developed rhizoids. Columellae, sporangia, chlamydospores, and zygospores are absent.

Upadhyay placed the genus in the Thamnidiaceae and suggested that it was related to Helicostylum, Cokeromyces, and especially to Radiomyces.

Both species of Radiomyces are homothallic, forming smooth-walled zygospores surrounded by long, flexuous, branched appendages borne on the opposed suspensors.

The genera are easily distinguished. Hesseltinella has a single, stalked, many-spored sporangiole borne on the secondary vesicle, whereas Radiomyces has many single- or multispored, stalked sporangioles borne
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on secondary vesicles. Radiomyces is homothallic, whereas Hesseltinella presumably is heterothallic.

LITERATURE CITED


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