Some interesting species of *Emericella* and *Aspergillus* from Egyptian desert soil

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SAMSON, R. A. and MOUCHACCA, J. 1974. Some interesting species of *Emericella* and *Aspergillus* from Egyptian desert soil. Antonie van Leeuwenhoek **40**: 121–131.

Emericella desertorum Samson et Mouchacca spec. nov. is described and illustrated. It differs from the other *Emericella* species by the large ascospores with low crests. An *Aspergillus* conidial state was not observed. Additional information on the morphology and physiology of *Emericella fruticulosa* (Raper et Fennell) Malloch et Cain and *Aspergillus egyptiacus* Moubasher et Moustafa is given. The osmophilic and halophilic properties occurring within the genus *Aspergillus* are discussed.

INTRODUCTION

In a preliminary survey of the fungus flora of soil samples collected in the Western Desert of Egypt, some interesting species of the genera *Emericella* and *Aspergillus* were encountered. One of the *Emericella* species proved to differ sufficiently from all known *Emericella* species to warrant its description as a new taxon. Another *Emericella* species was identified as *E. fruticulosa* (Raper et Fennell) Malloch et Cain, though it differs slightly from the type culture in its cultural appearance. A number of strains represent *Aspergillus egyptiacus*, recently described by Moubasher and Moustafa (1972). Because of the origin of the isolated strains special attention has been paid to their temperature relationship and their development on agar media with various sugar concentrations.

Emericella desertorum Samson et Mouchacca, spec. nov.

Coloniae in agaro Czapekii fere celeriter crescunt, post 14 dies 6–7 cm diam. 35 C, compacte intricatae multis ascomatibus; primum luteolae, deinde purpurascentes, violaceo-ardesiacae; reversum subluteum, deinde purpurascens, brunneum vel atrum.

Ascomata fere globosa, 100–150 μm diam., non ostiolata, denso strato hypharum intricatarum circumdata numerosis "Hülle"-cellulis plerumque globosis, 12.5–25 μm interspersis.

Initialia ascomatum e glomis compactis hypharum tenuium constant. Asci catenulati, globosi, 10.5–14.0 μm, 8-spori. Ascosporae continuae, aurantiacae, lenticulares, duabus cristis depressis praeditae, parte convexa levi vel minute asperata, 6.7–8 × 6–7.5 μm. Species osmophila.

Typus CBS 653.73, isolatus ex arena deserti in Aegypto.

Colonies on Czapek agar growing rather fast, attaining a diameter of 6 to 7 cm within 14 days at 35 C, consisting of a compact felt of numerous ascomata, occasionally covered by a scanty overgrowth of aerial mycelium; at first yellowish, but with the ripening of the ascomata quickly changing to purple shades near Dark Slate Violet (1) and (2) (Ridgway, 1912, Pl. 43 and 44). Aerial hyphae sparsely developed, smooth-walled, hyaline, 2.0–2.5 μm in diameter. Reverse yellowish at first, becoming purplish-brown to black. Exudate and odour inconspicuous or absent.

Ascomata more or less globose, 100–150 μm in diameter, non-ostiolate consisting of a wall of one layer of reddish purple cells, surrounded by a hyaline layer of scattered hyphae bearing numerous Hülle cells, mostly globose, 12.5–25 μm in diameter. Ascomatal initials developing as an irregular, compact clue of thin hyphae. Asci produced in chains, globose, 10.5–14.0 μm, 8-spored. Ascospores one-celled, orange-red, lenticular with two very low crests and with a convex wall roughened, when seen under light microscope, but nearly smooth-walled with only a few roughenings, when observed by means of scanning electron microscope (Fig. 1), 6.7–8 × 6–7.5 μm. At 25 C colonies growing somewhat slower, attaining a diameter of 4.5–5.0 cm within 14 days. On malt agar at 35 C colonies spreading broadly, attaining a diameter of 8 cm within 14 days, but colonies less compact and with more overgrowth of aerial mycelium.

The species is osmophilic, developing luxuriantly on Czapek agar + 20% and 70% sucrose and on malt agar + 20% and 40% sucrose. The perfect state is well developed on Czapek or malt agar + 20% sucrose; no ripe ascomata are produced on Czapek + 70% and malt agar + 40% sucrose. Optimal growth occurs at 35 C; minimum temperature 15 C; maximum between 40 and 45 C.

No conidial state was observed on all tested media.

Cultures examined:

CBS 653.73 = type culture; CBS 654.73 and CBS 655.73, isolated from a soil sample, collected from a grey soil at km 32 south of Kharga town near Kharga oasis. The strains were isolated on malt plates with various salt concentrations.

Emericella desertorum differs from all other species of *Emericella* by its much larger ascospores, which are ornamented with two low crests. This ornamentation resembles that of *Emericella nidulans* (Eidam) Vuill. var. *acristatus* (Fennell et Raper) Subram. Fennell and Raper (1955) described the ascospores of this variety as being crest-free, but observations by means of scanning electron

microscopy (Locci and Quaroni, 1971) showed that two low crests are present.

Since *Emericella* species are usually accompanied by an *Aspergillus* conidial state, the strains of *E. desertorum* were cultivated on various media and under different conditions to obtain its imperfect state. Despite numerous attempts no

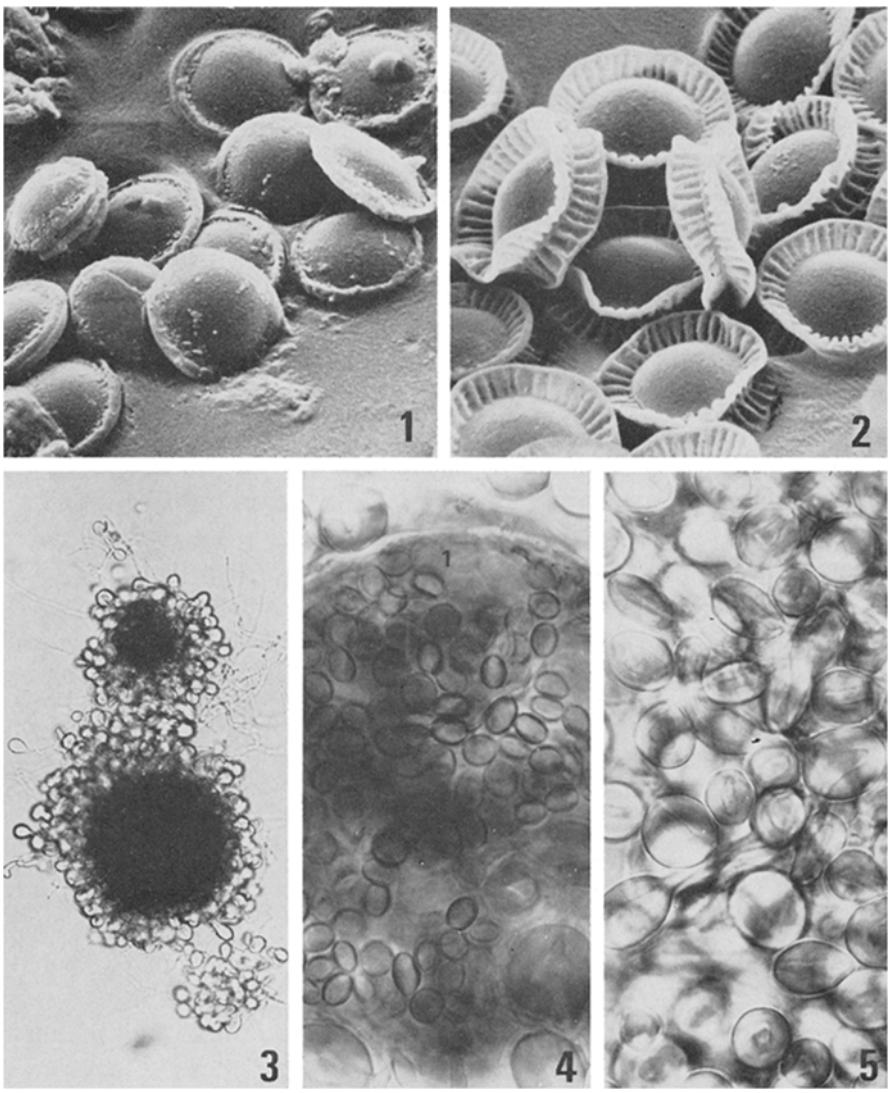

Fig. 1–5: 1–2 Scanning electron micrographs of ascospores 1. *Emericella desertorum* (2000 ×), 2. *Emericella fruticulosa* (3000 ×); 3–5 *Emericella desertorum* 3. ascomata (150 ×), 4. ascospores (800 ×), 5. Hülle cells (600 ×).

conidial state could be observed. Conidial structures in some species of *Emericella* (e.g. *E. striata*, *E. violacea*) may be also very limited in number. The new species is placed in *Emericella* because of its characteristic ascomata surrounded by abundant Hülle cells and the orange-red, lenticular ascospores.

Emericella fruticulosa (Raper et Fennell) Malloch et Cain in Can. J. Bot. **50**: 61 (1972).

Status conidialis: *Aspergillus fruticulosus* Raper et Fennell in "The genus *Aspergillus*", p. 506 (1965).

Malloch and Cain (1972*a*) transferred *Aspergillus fruticulosus* to the ascomycetous genus *Emericella* and published it as a new combination. In their studies on *Talaromyces* and related genera Stolk and Samson (1971, 1972) considered such combinations as not validly published according to their interpretation of Art. 59 of the International Code of Botanical Nomenclature (Stafleu, 1972) – the combination of the specific epithet of a name typified by an imperfect state with a name of a genus characterized by a perfect state shall be considered not validly published as a new combination but shall be considered the name of a new taxon–. Accordingly species of *Penicillium* described inclusive of their perfect state when transferred to *Hamigera* or *Talaromyces* were published as new species, thus leaving the *Penicillium* name for the conidial state. Malloch and Cain (1972*a*, *b*) and Subramanian (1972) considered the *Aspergillus* species described inclusive of their perfect state as perfect taxa and used these *Aspergillus* names as the basionyms of the new combinations. Thus no distinctive names are left for the conidial state. Both interpretations of Art. 59 are debatable and a new proposal seems therefore desirable to emend this article, concerning the nomenclature of fungi with a pleomorphic life cycle.

Colonies on Czapek agar, growing moderately well, attaining a diameter of about 3 cm within 14 days at 25 C, consisting of a tough basal felt producing conspicuous ascending yellow or colourless hyphae depending on the strain, giving the surface of the colonies either a bright yellow appearance near Strontian Yellow (Ridgway, Pl. 16) or a more silvery aspect near Glaucous or Corydalis Green (Ridgway, Pl. 41). Sporulation mostly abundant. Perfect state usually ripening within 14 days. Odour sweet, but not pronounced. Reverse purple to purple brown or at first yellowish, becoming yellow brown with age. Colonies at 35 C growing somewhat faster, attaining a diameter of 4.5 cm within 14 days.

Colonies on Czapek agar with 20% and 70% sucrose growing restrictedly but sporulating abundantly. Growth thinner than on Czapek agar. Perfect state usually not produced.

Colonies on malt agar at 35 C, similarly developed but spreading broadly, attaining a diameter of 7.5 cm within 14 days, consisting of a dense layer of conidial heads, intermixed with ascomata and ascending hyphae, sometimes overgrown by a loose, somewhat floccose aerial mycelium. Ascending hyphae usually encrusted with yellow granules, 2.5–5 μm in diameter, septate. Colonies at first yellow green near Pale Turtle Green (Ridgway, Pl. 32), later becoming more grey green near Pale Lumiere Green (Ridgway, Pl. 17). Odour sweetish. Exudate absent. Vegetative hyphae usually yellow encrusted, about 3 μm in diameter.

Ascomata more or less globose, 100–150 μm in diameter, occasionally up to 500 μm, distinct, covered by tufts of radiating hyphae, which are usually encrusted with colourless or yellow granules, surrounded by globose to subglobose Hülle cells, 10 to 15 μm in diameter, ripening within 14 days. Asci produced in chains, subglobose, 10–12 μm in diameter, 8-spored. Ascospores purple red, lenticular, smooth, with two conspicuously pleated equatorial crests, up to 1.5 μm wide, 4.5–5.5 × 3.5–4 μm (crests not included).

Conidial heads biseriate, radiate, becoming columnar. Conidiophores up to 60 μm in length and 2.5–4.5 μm in diameter, smooth-walled, brown. Vesicles globose to flask-shaped, 4.5–7.5 μm in diameter. Metulae 6.0–6.2 × 2.5–3.5 μm, mostly bearing 2 phialides. Phialides 4.5–6.5 × 2.0–3.0 μm. Conidia globose to subglobose, echinulate, greenish, 2.5–3.5 μm in diameter.

Colonies on malt agar + 20% or 40% sucrose spreading broadly, attaining a diameter of 8 cm within 14 days at 35 C, consisting of a layer of conidial heads, arising primarily from the agar surface, occasionally intermixed with yellow ascending hyphae, giving the colony a yellow green to dark green appearance between Courge Green and Elm Green (Ridgway, Pl. 17). Perfect state not produced. Conidia slightly larger than those on other media. Hülle cells usually absent or sparsely produced.

The species shows an optimal growth at 35 C; minimum temperature 15 C; maximum 45 C.

Cultures examined:

CBS 486.66, type culture isolated by G. F. Orr from soil in the Colorado Desert, South California, USA.

CBS 989.72, isolated from an arid soil of recent reclamation and cultivated with corn near Mut, Dakhla Oasis, New Valley Region, Western Desert, Egypt.

CBS 650.73 A, B, C and D, isolated from a soil sample, collected from a grey soil at km 32 south of Kharga oasis, Egypt.

The Egyptian strains differ from the type culture by their prominent yellow mycelium. However, small sectors with yellow mycelium are occasionally

observed in the type culture, when it is growing on Czapek agar. The cultural growth of the examined strains is similar on malt agar.

The yellow aerial mycelium produced in the Egyptian strains is reminiscent of that of *Aspergillus recurvatus*. This species was characterized by Raper and Fennell (1965) by the recurved conidiophores, yellow mycelium and the absence of Hülle cells. A re-examination of the type culture of *A. recurvatus* showed that Hülle cells are present, while the conidiophores are often straight. Like the cultures of *Emericella fruticulosa* the type culture of *A. recurvatus* grows well on agar media with a high percentage of sucrose and has its optimal temperature at 35 C. The resemblance in morphology of *A. recurvatus* with *A. fruticulosus* suggest their identity. However, more strains should be found, before it can concluded that *A. recurvatus* represents the imperfect state of *Emericella fruticulosa*.

Aspergillus egyptiacus Moubasher et Moustafa in Egypt. J. Bot. **15**: 153 (1972).

Colonies on Czapek agar at 25 C, growing restrictedly, attaining a diameter of about 2.5 cm within 14 days, consisting of a tough basal felt with a floccose overgrowth of aerial mycelium; at first white, later creamish and with age near Light Buff to Ochraceous Buff (Ridgway, Pl. 15) or showing purple shades in the centre of the colony near Dark Lavender (Ridgway, Pl. 44). Reverse yellowish, becoming yellow brown. Conidial structures abundantly produced, but not affecting the colony appearance. Conidiophores mostly not arranged in a typical *Aspergillus*-head, but phialides solitary or occurring in little groups along the conidiophores resembling *Penicillium*-like structures (Fig. 6, d-e). At 35 C colonies grow faster, producing more typical heads (Fig. 7, b-c).

Colonies on Czapek agar with 20% or 70% sucrose, growing moderately well, showing more yellow shades, but growth is thinner than on Czapek; characteristic conidial heads are produced abundantly.

Colonies on malt agar with 20% or 40% sucrose, spreading broadly attaining a diameter of 8 cm within 6 days at 35 C. Conidial heads arising primarily from the agar surface, forming a dense layer, giving the surface of the colony a light green appearance near Asphodel Green to Grape Green (Ridgway, Pl. 41), after one week usually covered by a tuft-like aerial mycelium changing the colour of the colony into greyish green near Glaucous (Ridgway, Pl. 41). Exudate absent. Reverse yellowish at first, becoming yellow brown. Conidial heads biseriate, small, at first producing conidia in columns, up to 250 μm long, later somewhat radiating. Conidiophores short, 20–35 × 3.7–5 μm hyaline, smooth-walled. Vesicles, globose to dome-shaped, usually 7–8.5 μm in diameter. Metulae 6–10 × 3–4 μm, bearing 2 to 3 phialides. Phialides flask-shaped with a

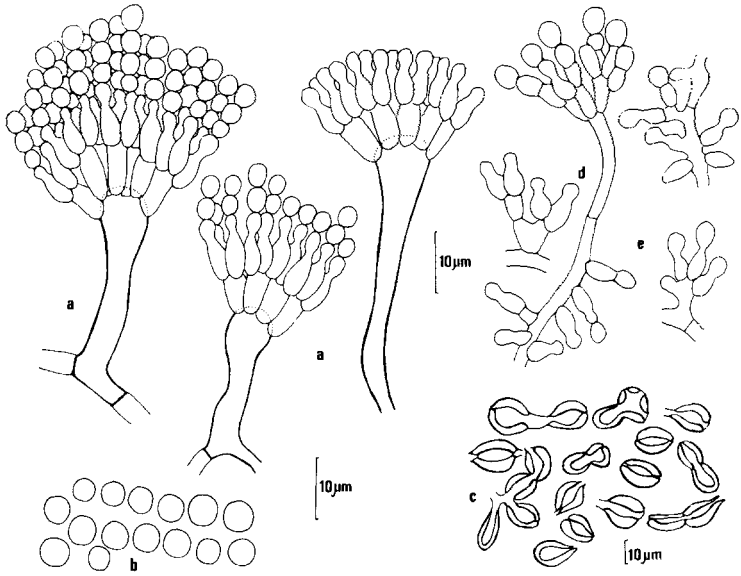

Fig. 6. *Aspergillus egyptiacus* a. conidial structures, b. conidia, c. Hülle cells, d–e. atypical conidial structures on Czapek agar at 25 C.

very short neck, 6–8.5 × 2.5–3.5 µm. Conidia globose, smooth, hyaline, 3.5–5 µm in diameter. Hülle cells sometimes absent, but mostly abundantly produced, irregular in form, varying from globose to almost cylindrical, 15–20 × 7–14 µm, often occurring in short chains. Perfect state not observed.

The species shows an optimal growth at 35 C; minimum temperature is 15 C; maximum 45 C. It is strongly osmophilic, growing even on Czapek agar with 70% sucrose.

Cultures examined:

CBS 656.73 = IMI 141,415, type culture of *Aspergillus egyptiacus*, isolated by A. H. Moubasher and A. F. Moustafa from sandy soil sample from olive-tree plantation in Ras-el-Hikma, Mediterranean coast, Egypt.

CBS 990.72, isolated from bare ferruginous soil, collected at km 32, Kharga-Beris road, New Valley region, Egypt.

CBS 991.72 A, 991.72 B, 991.72 C, 991.72 D, 991.72 E, 991.72 F all cultures isolated from bare ferruginous soil at km 17 near Mut, Dakhla Oasis, New Valley Region, Western Desert, Egypt.

In the strains CBS 991.72 B, CBS 991.72 D and CBS 991.72 F Hülle cells are absent or sparsely formed. The purple colour on Czapek agar is well developed in strains CBS 991.72 A and CBS 991.72 C, while in the other strains the colonies are yellowish.

Aspergillus egyptiacus can be best placed in the *A. nidulans*-group as redefined by Raper and Fennell (1965), because of the columnar conidial heads, the short conidiophores and the abundant production of Hülle cells. It has several characters in common with *A. subsessilis* Raper et Fennell. Both species were isolated from desert soil, though from two different continents. They produce short sessile conidiophores, globose conidia and atypical conidial heads on Czapek agar. *A. egyptiacus* can be distinguished from *A. subsessilis* by the slightly larger, smooth conidia and the smaller, irregular Hülle cells. Moreover the reverse of the colonies of *A. egyptiacus* shows yellow-brown colours, while that of *A. subsessilis* is usually purplish. In addition the strains of *A. egyptiacus* grow faster at 35 C, than those of *A. subsessilis*. Both species are osmophilic; colonies of *A. subsessilis* also grow on agar media with 20% to 70% sucrose.

A. egyptiacus resembles also *A. kassunensis* Baghdadi (1968), isolated from soil in Syria. The examination of type culture (CBS 419.69) of this species revealed a very scarce sporulation. The conidial heads have the same morphology and dimensions as those of *A. subsessilis*. Hülle cells are absent or sparsely developed, probably due to the degenerate state of the culture. Optimal growth occurs at 25 C. *A. kassunensis* is regarded as a possible synonym of *A. subsessilis*, but more additional strains have to be examined to verify this statement.

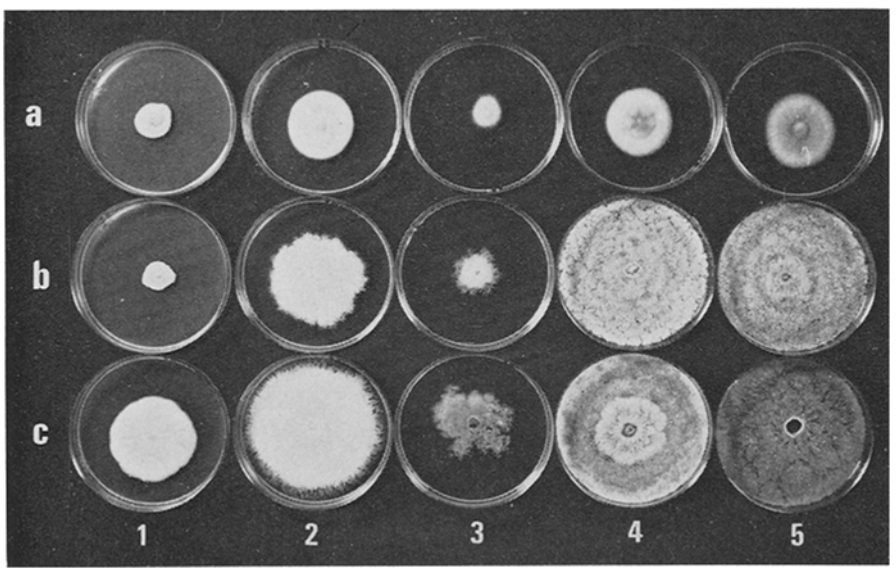

Fig. 7. *Aspergillus egyptiacus* and *A. subsessilis*. Colonies on agar media with various percentages of sucrose: Czapek (1), Czapek + 20% (2), Czapek + 70% (3), malt + 20% (4), malt + 40% (5). Series a: *A. subsessilis* at 25 C. Series b: *A. egyptiacus* at 25 C. Series c: *A. egyptiacus* at 35 C.

Another related species *A. crustosus* Raper et Fennell differs from *A. egyptiacus* by its long, pale-brown conidiophores, its rough conidia and the larger, globose Hülle cells. *A. crustosus* grows optimally at 35 C and shows also a strong osmophily.

Remarks on osmophily and halophily in the genus Aspergillus

Physiological adaptation to their environments is found to be frequently developed among the Aspergilli. Such factors may be of chemical kind e.g. nutrients and toxins, or physical e.g. temperature, moisture or osmotic pressure. The most pronounced adaptation is the ability to grow and sporulate in habitats characterized by low moisture levels and/or high osmotic pressure, often combined with high temperatures.

The groups of *Aspergillus* species delimited by Raper and Fennell (1965) bring together organisms possessing close ecological characteristics at the intra and inter group level. According to the literature, the *A. glaucus*, *A. restrictus* and *A. cremeus*-group are obligate osmophiles. Also the *A. ochraceus*, *A. niger*, *A. candidus*, *A. flavus*, *A. versicolor* and *A. terreus*-group comprise species, whose tolerance to high values of osmotic pressure has been proved. The ability to grow at higher temperatures is also present in species of the *A. fumigatus* and *A. terreus*-groups. Adaptation to a specific substrate, such as dung, is exhibited by the *A. clavatus*-group, the species of which are known for their ability to withstand strongly alkaline conditions.

Information concerning the ecology of the numerous species in the remaining groups is not yet available. Such is the case for the *A. nidulans*-group, which now comprises 19 species and 5 varieties, isolated from various origins, but mostly from soil. Some of them, although widely distributed in nature seem to occur most commonly in subtropical areas with a comparatively dry and warm climate.

A preferential occurrence in these areas suggests an adaptation to warm dry habitats. Cultural studies of a number of *Aspergillus* strains isolated from soil samples collected from the dry, warm New Valley region of the Western Desert of Egypt confirmed this hypothesis. The 3 species of the *A. nidulans*-group described in this paper grow optimally at 35 C, on media containing 40% sucrose, they are thus thermo- and osmotolerant. In addition, other members of the *A. nidulans*-group as *A. subsessilis*, *A. kassunensis*, *A. crustosus* and *A. recurvatus* have the same growth requirements.

Additional informations on the behaviour of a number of *Aspergillus* species presented in the last few years, consolidate the above generalisation for the genus. Kulik and Hanlin (1968) found optimal growth of *A. terreus* on media

containing 15% NaCl. Growth of *A. flavus*, *A. niger*, *A. ochraceus* and *A. parasiticus* was not much affected up to 10% salt concentration, but was strongly so by higher levels. The authors noted that strains of the same species respond differently to a given salt concentration. Mouchacca, Joly and Joly (1970) observed a gradual decrease in the linear growth of some Aspergilli with the increase in salt concentration of the medium. The rate of decrease in growth was not constant along the salt gradient used and varied with the species. Tolerance to salt decreased in the sequence *A. flavus* > *A. niger* > *A. puniceus* > *A. terreus* > *A. nidulans*. Sporulation, however, was not enhanced even at 2.0 M salt concentration except for *A. nidulans*, where cleistothecium formation was arrested at 0.8 M NaCl, while conidia were still produced at 2.0 M.

Aspergilli have also been found to be best adapted to activity at low moisture levels (Griffin, 1972) and are thus referred to as xerophilic organisms. Low moisture levels are obtained in the laboratory by media with increased concentrations of either sugar or with salt, and accordingly the organisms are regarded as "osmophilic" or "halophilic". Obligate osmophily is restricted to some groups of *Aspergillus*, the remaining ones are rather osmotolerant. Obligate halophily is unknown among the filamentous fungi. For the majority of "osmophilic" and "halophilic" species, the effect of NaCl or sucrose can be duplicated by other salts and non-electrolytes; these fungi are thus not so much "halophilic" in character as "osmophilic" or "xerophilic". Pelhate (1968) considered osmophily as being an aspect of xerophily; the ability of a fungus to support high osmotic pressures induced by higher concentrations of NaCl, sucrose or polyethylene glycol would allow a rapid and faithful evaluation of its xerophily.

A recent approach to the problem by measuring the water available in the substrate, the so-called water activity (a_w), has been developed (Scott, 1957). The water activity is the ratio of the vapour pressure of the water in the substrate to that of pure water at the same temperature and pressure. It allows a standardization of the terminology regardless of the nature of the water-limiting agent. Todd and Levitt (1951) measured the water bound in *A. niger* cultivated on media containing glucose. The bound water increased about tenfold as the glucose concentration increased. Additional information in this direction would no doubt be beneficial for a better understanding of the mechanisms operating during growth of fungi under selective and limiting physical factors of the habitat.

The authors thank Mr. D. Yarrow for correcting the English text and the Research Group of Scanning Electron Microscopy of the University of Amsterdam for their co-operation.

Received 21 *August* 1973

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