A revision of the genus Rhizopus
I. The Rhizopus stolonifer-group and Rhizopus oryzae

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SUMMARY

The genus *Rhizopus* Ehrenb. is revised, mainly using the strains maintained in the CBS culture collection. In the *Rh. stolonifer* group only two species are maintained, both with several varieties. The variable species *Rh. oryzae* is described. Numerous older taxa are treated as synonyms. Keys to the accepted taxa are provided.

Introduction

The genus *Rhizopus* Ehrenberg (1820) was based on *Rhizopus nigricans*, an incorrect name change for *Mucor stolonifer*, which was earlier described by Ehrenberg (1818). The first description is too concise for recognition, but the author’s figures of *Rhizopus nigricans* are distinctive. Vuillemin (1902) and Lind (1913) subsequently used the name *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill.

The early history of the genus was first reviewed by Zimmermann (1871). Possible synonyms of the type species were listed by Fischer (in Rabenhorst, 1892). Well known treatments of the genus are those of Lendner (1908), Hanzawa (1915), Zycha (1935), Zycha et al. (1969), Naumov (1935/1939) and Inui et al. (1965).

The genus *Rhizopus* would appear to be quite large judging from the number of species described. However, in the past species differentiation was often based on minor differences, e.g., the abundance of sporulation, or characters not currently used in the Zygomycetes, such as the ability to grow with or to ferment given components. Zycha (1935) and others reduced the number of species considerably.

The taxonomic decisions in the monograph of the genus by Inui et al. (1965) are not accepted here. Firstly, their use of the term “type-strain” is incorrect: several of these strains clearly do not originate from the authors of the species. Secondly, the use of keys combining morphological and physiological characters is not current in zygomycete taxonomy.

A need for a total review of the genus was felt, expanding the investigations of Scholer (1970) and Scholer and Müller (1971) on potentially human pathogenic species of *Rhizopus*, a large part of the accepted species. Throughout the present study, reference will be made to Scholer’s thorough investigation,
especially where dealing with the small-sized species, the *Rh. microsporus*-group.

**Material and methods**

For the present revision all available *Rhizopus* strains from the CBS collection were investigated: 82 strains, which had been received under 52 different names. The study mainly covered the general morphology. Light microscopy of water preparations was augmented with scanning electron microscopy (SEM) of spore ornamentation, particularly in the *Rh. microsporus*-group. Basic criteria for species delimitation were derived from mating experiments. Temperature relations were also established. Physiological characters were not included.

Species are mentioned as synonyms only where type strains were available. Several *Rhizopus* species described in not easily accessible Japanese journals were judged using diagnoses translated by Japanese colleagues.

The routine medium for morphological and growth studies was malt agar (MEA). For mating experiments various media were used: MEA: c. 3.5% glucose, PH 7; PDA: c. 2% glucose, PH 6.6; cherry decoction agar (ChA): PH 3.8–4.6; yeast extract agar (YEA): yeast extract 4 g, malt extract 10 g, glucose 4 g per liter, PH 7.3. Cultures were grown in 90-mm glass petri dishes containing 15 ml of nutrient.

Growth/temperature relations were established as radial growth (dishes centrally inoculated with identical pieces of expanding zone, 10 mm diam, placed upside down). Measurements were made after 24, 48 and 120 h.

The length of sporangiophores was taken from sporangiophores borne on stolons, not from single ones originating from an aerial hypha. The size of the sporangia was measured in undisturbed colonies with the aid of a stereomicroscope. Sporangiospore shape and size were determined in watermounts with light microscopy.

Sizes of sporangia, columellae and zygospores are given “up to” the largest of a continuous series. Rare extremes, not connected with the series by intermediate measurements are given between brackets (Schipper, 1975).

Sample preparation for SEM was after Samson et al. (1979).

Zygospore production was determined in colonies flooded with water, (a) to prevent the spreading of sporangiospores of potential human pathogenic species through the air, and (b) to dissolve sporangial walls so that zygospores were more easily recognized. If necessary the colonies were systematically flattened with an inoculation needle.

**Generic description and delimitation**

*Rhizopus* Ehrenb.


Sporangiophores mostly formed on stolons opposite rhizoids, either single
or more often in clusters, unbranched, occasionally divided near the top, bearing multisporated, terminal sporangia. Sporangia globose, distinctly columellate, apophysate, greyish to brownish at maturity. Sporangiospores (sub-)globose to ellipsoidal and angular. Zygospores covered with spines or warts, formed in aerial mycelium between non-ornamented, isogamous, opposite suspensors. Type species: *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill.

*Rhizopus* differs from *Mucor* Mich.:Fr. and *Phycomyces* Kunze:Fr. by having stolons and rhizoids. It can be separated from *Actinomucor* Shostakovich by dark-coloured sporangia on unbranched sporangiophores, mostly arising from well-developed stolons with distinct rhizoids, and from *Absidia* v. Tiegh. by its globose to hemisphaerical sporangia on unbranched sporangiophores opposite rhizoids. *Thermomucor* Subrahmanyam et al. has branched sporangiophores, and *Rhizomucor* (Lucet & Cost.) Wehmer ex Vuill. has non-apophysate sporan-

![Fig. 1. Schematic representation of Rhizopus groups. a. *Rh. stolonifer* group; b. *Rh. oryzae*; c. *Rh. microsporus* group.](image-url)
Table 1. Summary of diagnostic characters of *Rhizopus* groups.

<table>
<thead>
<tr>
<th>rhizoids</th>
<th>stolonifer-group</th>
<th>oryzae</th>
<th>microsporus-group</th>
</tr>
</thead>
<tbody>
<tr>
<td>sporangiophores</td>
<td>complex, well developed</td>
<td>medium</td>
<td>simple</td>
</tr>
<tr>
<td>sporangia</td>
<td>1-3 (4) mm long</td>
<td>max. 1-2.5 mm long</td>
<td>mostly up to 0.5 mm, rarely up to 1 mm</td>
</tr>
<tr>
<td>sporangia (150) 250-275 (300) µm in diam</td>
<td>max. diam 160-240 µm</td>
<td>up to 100 µm in diam</td>
<td></td>
</tr>
<tr>
<td>zygospores</td>
<td>black, up to 225 µm in diam</td>
<td>brown, up to 140 µm in diam</td>
<td>reddish brown, up to 90 (100) µm in diam</td>
</tr>
<tr>
<td>suspensors (appr.) equal</td>
<td>unequal</td>
<td>45°C</td>
<td></td>
</tr>
<tr>
<td>max. growth temp. (33) 36°C</td>
<td>over 45°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>occurrence, biology</td>
<td>on overripe fruit</td>
<td>food fermentors; agents of mucormycosis</td>
<td></td>
</tr>
</tbody>
</table>

**Key to the groups**

1. Sporangiophores mostly not exceeding 0.8 mm in height; rhizoids simple; sporangia up to 100 µm in diam; usually growth at 45°C
   *Rh. microsporus*-group

2. Sporangiophores often more than 1 mm in height; rhizoids with secondary branching; sporangia often over 100 µm in diam; no growth at 45°C
RHIZOPUS STOLONIFER-group

GENERAL CHARACTERISTICS AND VARIABILITY

Asexual structures. — Common features of the majority of the strains of the Rh. stolonifer-group are the following:

Rhizoids well developed. Sporangiophores mostly up to about 2000 µm in length, 20-25 µm diam; sporangia up to about 250 µm in diam; length of columellae slightly more than half the sporangial height, the larger columellae conical-cylindrical in shape, mouse grey or brownish; terms used for columella shapes are explained in Fig. 2. Sporangiospores angular-subglobose to ellipsoidal, with distinct ridges on the surface. No growth at 33°C.

Rhizopus stolonifer var. stolonifer and var. lyococcos (Ehrenb.) Stalpers & Schipper were described from 3-day-old petri dish cultures on MEA at 25°C. As Rh. sexualis (G. Smith) Callen did not show optimal development at 25°C, data on the strains of this species were derived from colonies grown at 20°C for 4 (var. sexualis) or 8 days (var. americanus Hesseltine & Ellis). The effect of temperature on viability of sporangiospores of both species was studied by Dennis and Blyham (1980), while the effect of humidity on formation of sporangiospores on strawberries was investigated by Harris and Dennis (1980).

The homothallic species Rhizopus sexualis resembles Rh. stolonifer in the shape and size of its sporangiospores, but differs in having relatively small sporangia (incl. columellae) and small sporangiophores. Rh. sexualis var. americanus has a somewhat different sporangial state, but should be regarded as a close relative because of similar zygosporic stages and temperature responses.

Zygospores. — The species of the Rh. stolonifer-group, both heterothallic and homothallic, produce black, relatively large zygospores. The ornamentation of the outer spore wall differs widely from that of the Rh. microsporus- and Rh.

Table 2. Occurrence of zygospores in contrasts of Rh. stolonifer. CBS 319.35 and 320.35 are strains of var. lyococcos. x = positive.

<table>
<thead>
<tr>
<th>CBS Nr.</th>
<th>320.35</th>
<th>263.28</th>
<th>403.51</th>
<th>442.74</th>
<th>107.76</th>
<th>609.82</th>
<th>853.72</th>
</tr>
</thead>
<tbody>
<tr>
<td>319.35</td>
<td>x</td>
<td>x</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>347.49</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>-</td>
</tr>
<tr>
<td>108.76</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>109.76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td>x</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>178.82</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>479.82</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td>x</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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oryzae-groups (Plate 1). Within the *Rh. stolonifer*-group morphological differences in the zygosporic stages are found in the size and shape of the suspensor pairs.

The first description of the zygosporic stage of *Rh. stolonifer* was given by de Bary (in de Bary & Woronin, 1866) in cultures on fleshy fruits and on bread, during a warm period in early summer. Several subsequent finds of zygospores were from spontaneous cultures on sweet potato, etc. Blakeslee (1904) demonstrated heterothallism in *Rh. stolonifer*.

In the present study zygospores (Fig. 3) were obtained on various media under very moist conditions, at 25–27°C. However, repeated contrasts under seemingly identical conditions were often unsuccessful. The use of small, tightly closed petri dishes (advised by Blakeslee, 1904) did not give notable improvement. In addition, the degree of development varied in successful matings of

![Fig. 3. Zygospores in matings of *Rh. stolonifer*. a. CBS 442.74 × 108.76; b. CBS 263.28 × 319.35; c. 442.74 × 109.76; d. 319.35 × 320.35; e. CBS 320.35 × 108.76.](image-url)
the same partners. Comparatively small and seemingly “unfinished” zygospores were often found; well-developed ones were comparatively rare. Even the largest, black zygospores were irregular, with frayed projections and slightly angular bodies. The addition of trisporic acid and methyl 4-dihydrotrisporate (both kindly supplied by Prof. Dr. H. v.d. Ende, University of Amsterdam) only gave a slight improvement. The occurrence of zygospores is summarized in Table 2. The tester pair was CBS 107.76 (−) and 108.76 (+).

Gauger (1977) germinated mature and immature zygospores as well as immature suspensors of mated Rh. stolonifer. From mature zygospores he obtained (+) or (−) colonies, from immature zygospores (+), (−) or (±) colonies; the latter segregated into (+) or (−), and recovered (+) colonies out numbered (−) colonies. Germination of immature suspensors yielded colonies of the parental or opposite genotype.

Namyslowski (1906) described a homothallic strain of Rh. stolonifer which produced zygosporic colonies from single vegetative spore isolations, if grown under favourable conditions. Many parthenospores and incomplete conjugations were also observed.

Rhizopus sexualis is a homothallic species, resembling Rh. stolonifer in several respects. Callen (1940) contrasted the type strain of Rh. sexualis with (+) and (−) strains of Rh. stolonifer and obtained “hybrid zygospores” in both cases, though most abundantly with Rh. stolonifer (+). Callen did not regard them as true hybrids but only as “mixochimeres with an association of partnership of nuclei.” Germination could not be obtained.

Schipper (1978) considered Callen’s “hybrid-zygospores” to be induced parthenospores. Induced parthenospores develop from one gametangium only, as lysis of the fusion-wall between gametangia does not occur; consequently these products need not indicate relationships or specific identities of the strains involved. Hawker and Syrop (1973) established that zygospore initiation and development in Rh. sexualis were poor at temperatures below 10°C. According to Harris and Dennis (1980) zygospore production is favoured at 90% relative humidity or above.

Key to the taxa

1 Zygospores present in single-sporangium isolates  
   Zygospores absent from single-sporangium isolates  

2 Sporangia more than 150 µm in max. diam  
   Sporangia less than 100 µm in max. diam  

   Rh. sexualis var. sexualis  
   Rh. sexualis var. americanus

3 Sporangiophores erect  
   Sporangiophores partly recurved  

   Rh. stolonifer var. stolonifer  
   Rh. stolonifer var. lyococcos
DESCRIPTIONS OF ACCEPTED TAXA

Rhizopus stolonifer (Ehrenb.:Fr.) Vuill. var. stolonifer


The description is of CBS 609.82:

Colony whitish, with sterile aerial mycelium and black spots of sporangia and dark sporangiophores. Rhizoids well developed. Sporangiophores (on stolons) up to 2000 × 20 μm, brown, in groups of 1–3 (occasionally more). Sporangia blackish, powdery in appearance, up to 275 μm in diam. Columellae conical, mouse-grey, up to 140 μm in height (rarely larger). Sporangiophores angular-globose-ellipsoidal, up to 13 μm in length, distinctly striate.

No growth at 33°C; growth and sporulation at 15–30°C.

**MATERIAL EXAMINED**

CBS 263.28 (—); CBS 322.35, P. Claussen, orig. G. Linnemann; CBS 347.49 (+), ex temphe, E.E. Siahajia; CBS 403.51 (—), Nagao Institute, Tokyo; CBS 853.72 (—); CBS 442.74 (—), ex coffee ground; CBS 107.76 (—), E. Müller No. 2828 = Scholer M-9; CBS 108.76 (+), E. Müller No. 2836 = Scholer M-79; CBS 109.76 (+), E. Müller No. 2675; CBS 609.82 (—), ex ragi; CBS 126.83, ex ragi; CBS 478.82 (+); CBS 479.82 (+); CBS 150.83 = IMI 62010, *Rh. artocarpi*, ex *Artocarpus integer*.

**DISCUSSION**

Strain variability: in CBS 108.76 rhizoids were rather small; CBS 442.74 failed to grow at 30°C; CBS 126.83 was almost devoid of rhizoids, poorly sporulating, with “unfinished” sporangiospores, poorly developed at 27°C and rather difficult to subculture.

CBS 403.51 was received from the Nagao Institute, Tokyo, as authentic for *Rhizopus niveus* Yamazaki. According its diagnosis (Yamazaki, 1919) *Rh. niveus* should be a small-sized *Rhizopus* species, but CBS 403.51 was large from the time it was received in 1951. Probably somewhere along the way an error has been made; the name *Rh. niveus* therefore remains doubtful.

*Rhizopus artocarpi* Racib. (1900) was described from the male inflorescence of *Artocarpus* sp. Raciborski found zygospores in one of his agar cultures, but never on the host from which the strains were isolated. The suspensors were described as equal in size, 90–120 μm in length, conical, widening towards the zygospore to 70 μm. The asexual state of the type strain, CBS 150.83, is indistinguishable from *Rh. stolonifer*; zygospores were not produced. *Rh. artocarpi* (Berk. & Br.) Boedijn is a later homonym based on *Mucor artocarpi* Berk. & Br. (1873). The description is very incomplete, no mention is made of zygospores. Boedijn (1958/59) described zygosporic isolates from *Artocarpus*, but did not
determine homo/heterothallism of the isolates. The “swollen” suspensors give an incompletely picture.

**Rhizopus stolonifer** (Ehrenb.:Fr.) Vuill. var. *lyococcos* (Ehrenb.:Fr.) Stalpers & Schipper


**Material Examined**

CBS 319.35 (+), CBS 320.35 (−), F. Zach; CBS 117.43.

**Discussion**

This variety is distinct from var. *stolonifer* by the presence of recurved sporangia and smaller sporangiospores. For a discussion of the synonymy of *Sporotrichum lyococcos* and *Rh. reflexus* see Stalpers (1983).

According to the first description of *Rhizopus reflexus*, all sporangiophores were recurved. It was noted that *Rh. reflexus* showed poorer growth than *Rh. stolonifer* in summer but better development in winter; this might point to a slight psychrophily in Bainier’s strain. No authentic material is known to be preserved. The above strains produce both recurved and erect sporangiophores and have temperature responses similar to those of *Rh. stolonifer*. The strains CBS 319.35, 320.25 and 117.43 produce colonies with both recurved and erect sporangiophores, under various conditions of temperature, humidity, light, medium and position (normal or upside down). Colonies originating from single sporangia produced sporangiophores of both types, independent of the condition of the parent sporangium.

Bainier (1882) noted in *Rh. reflexus* an inequality in thickness of the sporangiophore wall between inner and outer curvature. This seems to indicate a permanent condition and not just a transitional stage. In microscopic preparations an inequality of wall thickness was observed, though not restricted to the recurved area but also at alternating sides in straight parts of recurved and erect sporangiophores. This seems to indicate spiral growth comparable to that of, e.g., *Phycomyces blakesleeanus* Burgeff (Oort, 1931; Oort and Roelofsen, 1932) and *Zygorhynchus exponens* Burgeff (Grehn, 1932).

Zycha (1935) synonymized *Rh. circinans* v. Tiegh. (1876) and *Rh. reflexus*, after studying one of the Zach strains. According to the original diagnosis, *Rh. circinans* is a small-sized species of *Rhizopus* with strongly recurved sporangia. The available strains that show recurved sporangiophores are much larger, close to *Rh. reflexus*. *Rh. circinans* is of doubtful identity.
Rhizopus sexualis (G. Smith) Callen var. sexualis


The description is of CBS 336.39:

- Colony whitish (due to abundant sterile mycelium). Rhizoids few and small.
- Sporangiophores (on stolons) up to $1500 \times 20$ $\mu$m, brown; dichotomous top-branching occurs. Sporangia blackish, powdery in appearance, up to $175$ $\mu$m in diam, occasionally larger. Columellae conical-cylindrical, up to $100$ $\mu$m in height, mouse-grey. Sporangiospores angular-globose-ellipsoidal, up to $13(-15)$ $\mu$m in length, distinctly striate. Zygospores (homothallic) black, up to $150$ $\mu$m in diam, with blunt projections. Suspensors globose, equal or nearly so.
- No growth at $30^\circ$C; growth and sporulation at $15$–$25^\circ$C.

**MATERIAL EXAMINED**

CBS 336.39 (homothallic), type culture; CBS 123.64 = IMI 10348 (homothallic).

**DISCUSSION**

CBS 123.64 differs by the production of slightly larger zygospores (up to $170$ $\mu$m in diam) and optimal development at $15^\circ$C. The descriptions of the type strain by Callen (1940), Hesseltine and Ellis (1961) and the above all deviate from the original description by Smith (1939) in having larger sporangia and columellae and wider sporangiophores. The present CBS cultures do not show the described symmetrical, bifurcate zygophores.

**Rhizopus sexualis** (G. Smith) Callen var. americanus Hesseltine & Ellis


The type strain, CBS 340.62, differs from the var. *sexualis* by: colony irregular in outline; sporangiophores up to $700 \times 8$ mm; sporangia up to $75$ $\mu$m in diam, mostly smaller; columellae subglobose-applanate, up to $30$ $\mu$m in height; zygospores up to $225$ $\mu$m in diam.

**MATERIAL EXAMINED**

CBS 340.62 (homothallic), type strain of *Rh. sexualis* var. *americanus*.

**DISCUSSION**

CBS 340.62 sporulates poorly; the sporangial apparatus is poorly developed.

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While in strains of the var. sexualis zygospore production can be poor, in the var. americanus it is the prevailing character. The supposition that the production of abundant, large zygospores might cause a dwarfining of the sporangia was tested by trying to suppress zygospore production by means of light, change of medium and temperature, but without success. Zygospore production was strong under all conditions of this investigation.

**RHIZOPUS ORYZAE**

Most strains of *Rh. oryzae* were isolated as active components in the production of oriental foods or alcoholic beverages in Indonesia, China and Japan. Many hardly distinguishable species have been described in the older literature. A trend towards a reduction of the number of accepted taxa has become apparent since Zycha’s (1935) revision of the genus.

Inui et al. (1965) divided the genus into sections on the basis of temperature responses. Their section *Oryzae* consisted of species growing well at 37°C but not at 45°C in Pfeffer’s solution. Dabinett and Wellman (1973) used numerical methods on data published by Inui et al. (1965) and arrived at an essentially similar classification.

Scholer (1970) regarded the species available for the present study all as synonymous with *Rhizopus oryzae*, though in a number of species the identity could not be established with certainty. His conclusion is confirmed in the present study, based on a detailed analysis of the morphology, and on mating experiments.

**GENERAL CHARACTERISTICS**

Common features of most strains of *Rh. oryzae* were: rhizoids medium sized (see Fig.1), sporangiospores up to 1000-1500 μm in length, (10—)13—15(-20) μm in width, local swellings and dichotomous branching were present. Sporangia up to 150-175 μm in diam. Columellae ellipsoidal on a truncate base, mouse-grey or brownish. Sporangiospores angular, subglobose to ellipsoidal, with ridges on the surface, up to 8(-10) μm in length. No growth at 45° C, growth at 40° C.

**Zygospores.** — Namyslowski (1906) and Lendner (1908) mentioned the occurrence of zygospores in cultures of the type strain of *Rh. nodosus*. These were dark brown, av. 120-140(-180) μm in diam, with conical projections and borne between equal or unequal suspensors. Homothallic zygospores were not observed during the present study in monospore isolates of CBS 127.08, which is probably Namyslowski’s strain.

The mating partners VKM F-773(+) (= CBS 699.68) and VKM F-774(-) (= CBS 700.68), first designed as *Rhizopus oryzae* by A.A. Milko, were reidentified by Scholer (1970) as *Rh. microsporus*. A number of strains were contrasted with the above tester pair of *Rh. microsporus*, with the aim of elucidating mating abilities in *Rh. oryzae*. One response was observed: CBS 346.36 × CBS 700.68 (−) resulted in short lived, incomplete mating reactions. This determines *Rh. oryzae* CBS 346.36 as (+).
In random test contrasts within the *oryzae*-group, zygospor es were obtained in CBS 346.36 × CBS 127.08. YEA at 30°C was found to be the optimum for zygospore production. The zygospor es were reddish brown when young, then brown, stellate with conical projections, up to 140 μm in diam, between unequal suspensors. Subsequently, all other strains were contrasted with CBS 346.36 (+) and CBS 127.08 (−). Zygospores were obtained in CBS 346.36 × 112.07; 127.08; 110.17; 148.22; 257.28; 264.28; 266.30; 329.47; 382.52 and 285.55.

**Rhizopus oryzae** Went & Prinsen Geerl.


The description is of CBS 112.07:

Colony greyish brown. Rhizoids brownish. Sporangiophores on stolons up to 1500 μm in length, occasionally longer, up to 18 μm in width, with local
swellings, brown, single or aggregated in small groups. Sporangia greyish black, powdery in appearance, up to 175 μm in diam. Cilium ellipsoidal on a truncate base, up to 130 μm in height, mouse-grey. Sporangiospores angular, subglobose to ellipsoidal, with ridges on the surface, up to 8 μm in length. Zygosporae (112.07 × 346.36 on YEA at 30°C) reddish brown when young, then brown, stellate conical projections, up to 140 μm in diam; suspensors unequal.

No growth at 45°C; good growth at 40°C.

MATERIAL EXAMINED

CBS 112.07, type culture of *Rhizopus oryzae*; CBS 127.08, probably authentic for *Rh. nodosus*, P. Lindner; CBS 128.08, authentic for *Rh. tritici*; CBS 120.12, type culture of *Rh. delemar*; CBS 110.17, type culture of *Rh. maydis*; CBS 147.22, L. Harter; CBS 148.22, F. McFarland; CBS 256.28, B. Koehler; CBS 257.28, type culture of *Rh. formosaensis*, CLMR; CBS 258.28, type culture of *Rh. hangchao*, CLMR; CBS 259.28, CLMR; CBS 260.28, type culture of *Rh. liquefaciens*, CLMR; CBS 264.28, type culture of *Rh. pseudo-chinensis*; CBS 266.30, type culture of *Rh. fusiformis*; CBS 295.31, type culture of *Rh. suinus*; CBS 296.31, W. Butkewitsch; CBS 385.34, type culture of *Rh. achlamydoспорus*; CBS 386.34, type culture of *Rh. bahniensis*; CBS 387.34, type culture of *Rh. batatas*; CBS 389.34, type culture of *Rh. chinian* var. *isofermentarius*; CBS 390.34, type culture of *Rh. delemar* var. *minimus*; CBS 391.34, type culture of *Rh. javanicus*; CBS 393.34, type culture of *Rh. peka*, R. Nakazawa; CBS 395.34, type culture of *Rh. samarangensis*; CBS 321.35, *Rh. kasanensis*, G. Linnemann; CBS 324.35; CBS 346.36, L.H. Leonian; CBS 278.38, Boulard S; CBS 279.38, type culture of *Rh. somtii*; CBS 327.47 = ATCC 4858 = ATCC 4859; CBS 328.47 = ATCC 9374, A.F. Blakeslee; CBS 329.47 = ATCC 10260; CBS 348.49, K.B. Boedijn and J. Reitsma; CBS 401.51 = NI 1204; CBS 402.51, type culture of *Rh. javanicus* var. *kawasakiensis*; CBS 404.51 = NI 1206; CBS 405.51, type culture of *Rh. usami*; CBS 381.52 = ATCC 11145, H.C. Murray; CBS 382.52 = ATCC 6227, A.F. Blakeslee; CBS 330.53 = IFO 5318, type culture of *Rh. boreas*, Y. Yamamoto; CBS 395.54, L. Ajello; CBS 285.55; CBS 286.55; CBS 264.60; CBS 372.63; CBS 607.68, H. Frank.

DISCUSSION

The influence of external conditions was tested using CBS 112.07. It was grown on MEA, CMA and ChA at temperatures between 15 and 36°C with intervals of 3°C. At 27°C development was optimal on all media applied. At 36°C growth was rapid, but after 4 days the relatively short sporangiophores frequently showed large swellings, and sporangia were often defective with subglobose to conical colomella; complete sporangiospores were few and rather small. This effect was most pronounced on MEA. At 33°C after 4 days development was slightly better. At lower temperatures expansion growth and sporulation decreased. After 17 days at 15°C few swellings occurred in the sporangiophores. Sporangiphores and sporangia were found to be smaller. Of the three media, colonies on MEA were most sensitive to unfavourable temperature conditions with regard to sporulation. Usually the maximum growth temperature was reached abruptly; minimum temperatures were not determined.

The additional strains of the species showed variability in all mentioned characters. Rhizoids, usually abundant, were rare in a few strains. Sporangiphores were found to vary from 750 to 1500 (2000) μm in max. length. Sporangia varied from 100 (125) to 200 μm max diam. Cilium ellipsoidal in shape were not found in all strains; in a number of strains the larger collumella were subglobose.
to conical. Sporangiospores were found to vary between 6 and 10(-18) \( \mu \text{m} \) in max. length. The great majority of the strains grew well at 40°C, though occasionally the turf was rather low. A few strains deviated in their temperature response. CBS 381.52 did not grow at 36°C and produced a flat colony at 33°C; it is unknown whether the strain, which was used for oxidation of steroids, has been genetically manipulated. CBS 259.28 produced very small colonies at 40 and 36°C, growth was good at 33°C; the strain sporulated poorly, and sporangiospores were variable in size. CBS 390.34 grew slowly at 40°C, while good growth occurred at 36°C; CBS 386.34 showed slightly abnormal hyphae at 40°C; the strain sporulated poorly, sporangiospores being variable.

**Synonymy.** — Though *Rh. arrhizus* (Fischer in Rabenhorst, 1892) was described prior to *Rh. oryzae* (Went and Prinsen Geerligs, 1895), Scholer (1970) preferred the latter name. In his opinion, *Rh. arrhizus* was an extreme form of *Rh. oryzae*, showing applanate columellae which are very unusual in *Rh. oryzae*. Some support may be found in Hagem's (1907/08) description of his *Mucor arrhizus*, which was stated to be very similar to Fischer's isolate, also producing applanate columellae on MEA at 16°C, but globose to elongate columellae on bread at 22°C. However, Fischer's description is not conclusive, illustrations or type material are lacking, and therefore the species is treated as doubtful.

Vuillemin (1906) differentiated *Rh. japonicus* from *Rh. oryzae* on the basis of the absence of growth in Rosalin-solution at 37°C. On potato agar at 39°C growth occurred, though poorer than in *Rh. oryzae*. Further there were slight differences in growth rate, sporulation and colony appearance, and slightly larger sporangiospores: in *Rh. japonicus* mostly 9 \( \mu \text{m} \) in length, in *Rh. oryzae* about 6–8 \( \mu \text{m} \). Similar features were used by Vuillemin (1902) to differentiate *Rh. tonkinensis* from *Rh. oryzae* and *Rh. japonicus*, mainly by less development at higher temperatures. Sporangiospores of *Rh. tonkinensis* averaged 8 \( \mu \text{m} \) in length and the size of the sporangia was noted as 75(–100) \( \mu \text{m} \) in diam. In *Rh. japonicus* sporangial diameters were stated to be 160–215 \( \mu \text{m} \). The diagnosis of *Rh. oryzae* mentions sporangia up to 175 \( \mu \text{m} \), “in case of good nourishment”. Now that the wide range of variability of *Rh. oryzae* is better known, the above species can easily be reduced to synonymy.

According to Saito (1904), *Rh. tritici* differs from *Rh. oryzae* in the occurrence of swellings in the sporangiophores and confluent sporangiospores. The type strain, CBS 128.08, was studied by Hanzawa (1915). There are also notes available from the CBS files which prove that it is still in the original condition.

Hagem (1907/08) described *Mucor norvegicus*, isolated from soil in Norway, and deposited a culture at the CBS, but this is now lost. Lendner (1908) compared *Rh. nodosus* with this strain and found them to be identical. This was later confirmed by Hagem (1910). Lendner’s (1908) drawings of infundibuliform columellae are reminiscent of the “applanate” columellae described by Fischer (in Rabenhorst, 1892) in *Rh. arrhizus* and by Hagem (1907/08) in the synonymous *Mucor arrhizus* grown at low temperatures.

The type culture of *Rh. batatas* now deviates from older descriptions in producing sporangiospores up to 6–7 \( \mu \text{m} \) in length (MEA, 25°C): Nakazawa (1909) noted sporangiospores 4.4–12.3 × 3.5–5.2 \( \mu \text{m} \), and Hanzawa (1915) 4.2–9 × (3—)4.2–7.2 \( \mu \text{m} \).

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The morphological and physiological characterization of Rh. delemar was dated March 1912, but as soon as September 1912 a paper was presented by Hanzawa in which he remarks on the similarity of Rh. delemar and Rh. oryzae: the former might be just a more profusely sporulating strain of the latter. The type culture of Rh. delemar still fits the original diagnosis. Ellis (1981) investigated the influence of temperature, medium and age on the size of sporangiospores in colonies originating from a single sporangiospore of Rh. delemar (most probably the type strain). He showed that both medium and temperature have a great effect on sporangiospore size; the age of the colony much less so. The type strain of Rh. delemar var. minimus differs from that of the typical variety, CBS 120.12, in having shorter sporangiophores, smaller sporangia and columellae and the occurrence of variable sporangiospores, some large and partly misshapen.

Rhizopus kasanensis was mentioned prior to its diagnosis by Trubin (1911) as a pathogen on the eyes of rabbits. The taxon was later described twice by Hazawa (1912, 1915), and though the type culture is lost, these publications are sufficiently clear to decide on its synonymy with Rh. oryzae.

Hanzawa (1915) distinguished Rh. usamii from the related Rh. tritici, Rh. tonkinensis and Rh. batatas on the basis of colony colour, slight differences in sporangiospore size and minimum temperature for sporulation, Rh. usamii sporulating at lower temperatures (8–10°C). The probable type strain, CBS 406.51 was indistinguishable from Rh. oryzae.

CBS 257.28, sent-by the CLMR (Central Laboratory, South Manchuria Railway Co., Dairen) is probably authentic for Rh. formosaensis. Morphologically it is just another “medium-Rhizopus”; no distinguishing features are present.

Bruderlein (1917), who never before had come across poorly sporulating Mucor and Rhizopus strains, regarded the scarcity of sporangiophores and sporangia in his Rh. maydis as a major difference with known species. The type culture, CBS 110.17 developed better on CMA at 30°C than on MEA at 25°C, producing sporangia up to 150 μm in length and ellipsoidal columellae. The latter were illustrated in Bruderlein’s (1917) paper as subglobose.

CBS 401.51 was sent to the CBS by K. Kominami (Nagao Institute, Tokyo) as Rh. chungkuoensis with a reference to the diagnosis, but without a clear statement that indeed the type strain was concerned. It had slightly shorter sporangiophores (800–1000 μm), but in its remaining characters the strain is indistinguishable from Rh. oryzae.

CBS 258.28, the type strain of Rh. hangchao was sterile when this study was started. The type culture of Rh. liquefaciens, CBS 260.28 sporulated rather poorly on MEA, but much better on CMA. On the latter medium the sporangia were up to 175 μm and the sporangiospores 5–8(–10) μm.

The type strain of Rh. pseudochinensis, CBS 264.28 produced (MEA, 25°C) sporangiophores measuring 1000(–1500) × 13(–15) μm, sporangia 175 μm in diam, with subglobose to ellipsoidal columellae, up to 90 μm in height, and sporangiospores up to 8 μm in length. That of Rh. shanghaiensis, CBS 404.51 has not changed since its receipt at the CBS in 1951, and produces sporangiophores up to 800(–950) μm and sporangiospores up to 8 μm in length.

CBS 393.34, the type strain of Rh. peka deviates from the diagnosis in producing slightly longer sporangiophores and slightly larger sporangia.
A study of CBS 405.51, type strain of *Rh. thermosus*, as well as careful analysis of the original description, showed that just another *Rh. oryzae* was concerned. Also CBS 330.53, the type strain of *Rh. boreas* represents the same species, though with rather short sporangiophores, up to 800 µm, and small sporangia, up to 125(–150) µm.

According to Dawson and Povah (1928) *Rh. fusiformis* is characterized by cottony mycelium, sparse production of sporangia and branched sporangiophores with a fusiform swelling at the point of attachment of lateral branches. On MEA at 25°C the type strain, CBS 266.30, produces sporangia up to 175 µm in length instead of up to 113 µm as stated in the diagnosis. Judging from protologue (Nielsen, 1929), *Rh. suinus* is a micro-*Rhizopus*. However, CBS 295.31, the probable type strain, is closer to *Rh. oryzae*. Scholer and Müller (1971) too considered the species to belong in micro-*Rhizopus*, and CBS 295.31 an atypical strain of *Rh. oryzae*.

CBS 385.34, type culture of *Rh. achlamydosporus* differs slightly from the majority of *Rh. oryzae* strains by producing shorter sporangiophores, some of which with inconspicuous swellings, and rather large sporangiospores.

The type strain of *Rh. bahrnensis*, CBS 386.34, produces on MEA at 25°C sporangiospores up to 10(–12) µm in length, which is shorter than is usual in the group. On CMA at 30°C a lower incidence of narrow spores is found.

The diagnosis of *Rh. chiuniang* (Yamazaki, 1919) was not available to the present author. The paper also included a variety *isofermentarius*, but it could not be ascertained whether this was classified in *Rh. chiuniang*. The type strain, CBS 389.34, is of the common medium-*Rhizopus* type. The authentic material of *Rh. chiuniang* is probably lost. Takeda (1935) described *Rhizopus chungkuоensis* var. *isofermentarius* nov. var., with the following key-features: sporangia (39.9—)80–110(–199.5) µm, sporangiophores (186.2–)400–800(–1396.5) x (8.8–)9–10(–12.1) µm, columellae (35–)45–55(–70) µm, sporangiospores (5.5–)7–8(–11.5) x (3.8–)5–6(–6.6) µm; growth was observed at 40°C but not at 45°C.

CBS 391.34, type culture of *Rh. javanicus*, is identical to *Rh. oryzae*, except for the very variable sporangiospores, which are up to 8(–16) µm in length.

Takeda and Takamatsu (1949) described *Rh. javanicus* var. *kawasakiensis* as differing from the typical variety in growing with inulin, ammonium nitrate and glycocoll, and in its saccharification ability. In the type strain, CBS 402.51 few rhizoids were produced, which made it rather difficult to determine sporangiophore length. In the 1951 CBS files the sporangiophores were stated to measure 855–1425 µm in length; in 1982 they were up to 750 µm in length.

CBS 395.34, most probably the type strain of *Rh. semarangensis*, was almost sterile, but some sporulation could be induced on CMA at 30°C. Sporangiophores were very short (up to 250 µm) and sporangia small (up to 80 µm); columellae were semi-globose, sporangiospores variable, up to 10 µm in length, and chlamydospores abundant.

The diagnosis of *Rh. sontii* could not be traced. Reddi and Subrahmanyan (1935) stated that this rice-fermenting fungus had been provisionally described in their first publication. It would be allied to *Rh. cambocljae*, but differed in more pronounced physiological activities. The type culture, CBS 279.38 is a

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Rh. oryzae with variable sporangiospores, up to 8 (–14) µm in length. The Rh. cambodjae material is probably lost, and the concise protologue does not allow proper identification.

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