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# A taxonomic Study of the Genus *Rhizopus* Ehrenberg 1820*

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The genus *Rhizopus* Ehrenberg 1820 belongs to the most important technically used geni. It ferments various sugars, produces technically important acids, many growth factors and toxic substances and often parasites on plants and animals. It is therefore often isolated from nature, from fermenting liquids and in hygienical and sanitary research work after which it is usually identified. Some species of the genus *Rhizopus* have been described by Niethammer (1933) in a work on the systematic research of the soil in Bohemia and somewhat later by Dyr (1937—38, 1939, 1941). The identification of the findings in the soil of Bohemia was principally done according to an older paper of Hagem (1908) and a newer paper of Zycha (1935). The monograph of Zycha (*Mucorineae*, Kryptogamenflora der Mark Brandenburg, Pilze II, Leipzig 1935) compiles all the species of the genus *Rhizopus* described until that time, while in the papers of Niethammer and Dyr only those species of this genus are described, which have been found in Bohemia. In the work of Zycha there are described 54 species mentioned in various papers. These he divided in 8 main species according to their characterristic marks, e. g. the size of the spores, sporangia, sporangiophores, the surface and shape of the spores, the optimal temperatures for growth and the formation of gemmae. During the years 1942—45 we had the opportunity to work with many of the species described in the paper of Zycha as main species or as their synonyma. At the consideration whether our species belong to the genus *Rhizopus* Ehrenberg 1820 we have been led by the characteristic defined by Zycha:

*Rhizopus* Ehrenberg 1820 forms a richly branched mycelium with brownish or nearly black sporangiophores. These sporangiophores are most often formed on places, where the mycelium forms root-like formations, called rhizoids. Hyphae, from which these rhizoids grow are simple, long, broader then normal hyphae and are called stolons. The sporangia of this genus are big with hemispherical columella with a wide base, called apophysis. After the release of spores from the sporangia there does not remain a collar under the columella as is the case in the genus *Mucor*. The eight species described by Zycha are characterised by the above mentioned properties and are distinguished from one another by help of the following system:

1. Spores 3—4 $\mu$ long ..... *Rh. microsporus* van Tieghem 1875
   Synonyma:
   *Rhizopus minimus* van Tieghem 1875
   *Mucor speciosus* Oudemans 1902
   *Rhizopus equinus* Constantin and Lucet 1903
   *Rhizopus speciosus* (Oud.) Lendner 1908

   Spores longer ........................................ (2)
2. Sporangia overhanging *Rh. circinans* van Tieghem 1876
   Synonyma:
   *Rhizopus reflexus* Bainier 1882

* The experimental work was done at The Institute of Vitamine and Hormone Chemistry in Prague.

Sporangia on upstanding sporangiophors ................. (3)

3. Spores streaked .............................................. (4)
   Spores thickly thorny . . *Rh. echinatus* van Tieghem 1876

4. Sporangiophors and rhizoids delicate, maximally 1 mm high, optimal growthtemperature 30° C ...................... (5)
   Sporangiophors and rhizoids strong, even 2—4 mm. high ... (7)

5. Spores 7—12 $\mu$ long. . . . *Rh. oligosporus* Saito 1905
   Synonyma:
   *Rhizopus delemar* (Boidin) Wehmer and Hanzawa 1912
   *Rhizopus tamari* Saito 1907
   Spores 5—7 $\mu$ long .......................................... (6)

6. Sporangiophors longer than 150 $\mu$ .......... *Rh. arrhizus* Fischer 1892
   Synonyma:
   *Rhizopus nodosus* Namyslowski 1906
   *Rhizopus ramosus* Moreau 1913
   *Rhizopus maydis* Bruderlein 1917
   *Mucor arrhizus* (Fisch.) Hagem 1908
   *Mucor norvegicus* Hagem 1908
   *Rhizopus pusilus* Naumov (?)
   *Rhizopus chinensis* Saito 1904
   *Rhizopus bovinus* van Beyma 1931
   *Rhizopus cambodja* (Chrzaszcz) Vuillemin 1902
   *Rhizopus tritici* Saito 1904
   *Rhizopus chinensis* var. *rugosporus* Nakazawa 1913
   Sporangiophors smaller than 150 $\mu$ ........... *Rh. cohnii* Berlese and de Toni 1888
   Synonyma:
   *Rhizopus suinus* Nielsen 1929

7. Spores 7—9 $\mu$ long, abundant growth at 37° C and good formation of gemmae .....
   *Rh. oryzae* Went and Prinsee Geerling 1895
   Synonyma:
   *Rhizopus japonicus* Vuillemin 1902
   *Amylomyces beta* (Boidin) Zycha 1935
   *Amylomyces gama* Boidin 1901
   *Rhizopus tonkinensis* Vuillemin 1902
   Spores 10—12 $\mu$ long, growth at 37° C absent, gemmae formation .....
   *Rh. nigricans* Ehrenberg 1818, 1820
   Synonyma:
   *Mucor stolonifer* Ehrenberg 1818
   *Rhizopus niger* Ciaglinski and Hewelke 1893
   *Mucor niger* Gedoelst 1902
   *Rhizopus arthocarpi* var. *luxurians* Schröter 1886

In this system Zycha (1935) compiles only those strains, the descriptions of which were available to him. He barely mentions those strains abof which he did not have original descriptions:

*Rhizopus acidus* Yamazaki
*Rhizopus chungkuoensis* Yamazaki
*Rhizopus hangchao* Yamazaki
*Rhizopus kasanensis* Hanzawa
*Rhizopus niveus* Yamazaki
*Rhizopus salebrosus* Yamazaki
*Rhizopus trubini* Hanzawa
*Rhizopus apiculatus* Mac Alpine
*Rhizopus pigmaeus* Naumov
*Rhizopus umbellatus* Smith
*Rhizopus chinniang* Yamazaki
*Rhizopus formosaeensis* Nakazawa
*Rhizopus humilis* Yamazaki
*Rhizopus liquefaciesns* Yamazaki
*Rhizopus pseudochinensis* Yamazaki
*Rhizopus shanghaiensis* Yamazaki
*Rhizopus usamii* Hanzawa
*Rhizopus fusiformis* Dawson and Povah
*Rhizopus schizans* Mac Alpine

In more comprehensive studies of pure cultures of strains of the genus *Rhizopus* it was found that the sizes of organs of these strains are rather variable. In the investigation of our strains by help of the characteristics of Zycha we have found, that they often differ from the original description.

Our opinion about these differences is, that they are caused by minute differences of the cultivating conditions. The cultivating conditions of the strains, on which the original description was made, is often not described or cannot be described in all details. For the cultivation of this genus natural substrates (bread, carrot, worth) are most frequent and these substrates cannot be described in all details, so that they of course cannot be reproduced with absolute precision. It is also probable, that by many years of cultivation on artificial medium the original morphological characteristic of the studied strains somewhat have changed.

## Material and methods

A. Strains and their origin:

| Our designation | Species | Origin |
|---|---|---|
| SR 2 | *Rh. japonicus* Vuillemin 1902 strain No. 2 | A Niethammer, Prague 1942 |
| SR 4 | *Rh. japonicus* Vuillemin 1902 strain No. 4 | A. Niethammer, Prague 1942 |
| SR 5 | *Amylomyces beta* Boidin 1901 | Unknown |
| SR 6 | *Rh. tritici* Saito 1904 strain No. 1 | A. Niethammer, Prague 1942 |
| SR 7 | *Rh. tritici* Saito 1904 strain No. 2 | A. Niethammer, Prague 1942 |
| SR 8 | *Rh. tonkinensis* Vuillemin 1902 strain No. 1 | Zycha, Hannover Münden 1939 |
| SR 9 | *Rh. tonkinensis* Wuillemin 1902 strain No. 2 | Zycha, Hannover Münden 1939 |
| SR 10 | *Rh. tonkinensis* Vuillemin 1902 strain No. 3 | Manchuria Railway 1939 |
| SR 11 | *Rh. tonkinensis* Vuillemin 1902 strain No. 4 | Manchuria Railway 1939 |
| SR 12 | *Rh. chinensis* Saito 1904 strain No. 1 | A. Niethammer, Prague 1942 |
| SR 13 | *Rh. chinensis* Saito 1904 strain No. 2 | A. Niethammer, Prague 1942 |
| SR 18 | *Rh. delemar* (Boid.) Wehmer and Hanzawa 1912, strain No. 1 | Král Collection, Vienna 1939 |
| SR 20 | *Rh. delemar* (Boid) Wehmer and Hanzawa 1912, strain No. 2 | Král Collection, Vienna 1939 |
| SR 21 | *Rh. delemar* (Boid.) Wehmer and Hanzawa 1912, strain No. 3 | Unknown |
| SR 22 | *Rh. nigricans* Ehrenberg 1818 strain Kaki | Manchuria Railway 1939 |
| SR 23 | *Rh.* species Nill 1927, strain No. 1 | Král Collection, Vienna 1939 |
| SR 24 | *Rh.* species Nill 1927, strain No. 2 | Král Collection, Vienna 1939 |
| SR 26 | *Rh. chinniang* Yamazaki var. *isofermentarius* Cawamori (?) | C. B. S., Baarn |
| SR 27 | *Rh.* species Tanaka? | Manchuria Railway 1939 |
| SR 30 | *Rh.* species Takeda? | Manchuria Railway 1939 |
| SR 31 | *Rh.* species Lembke (?) strain No. 1 | Lembke, Kiel 1939 |
| SR 32 | *Rh.* species Lembke (?) strain No. 2 | Lembke, Kiel 1939 |
| SR 33 | *Rh. acidus* Yamazaki (?) | Manchuria Railway 1939 |
| SR 34 | *Rh. circinans* van Tieghem 1876 strain No. 1 | A. Niethammer, Prague 1942 |
| SR 35 | *Rh. circinans* van Tieghem 1876 strain No. 2 | A. Niethammer, Prague 1942 |
| SR 37 | *Rh. niger* Ciaglinski a Hewelke 1893 | Denk, Prague 1941 |
| SR 38 | *Rh. reflexus* Bainier 1882, strain No. 1 | Král Collection, Vienna 1939 |
| SR 39 | *Rh. reflexus* Bainier 1882 strain No. 2 | Král Collection, Vienna 1939 |
| SR 40 | *Rh. species* Takeda (?) strain Peka 2 | Manchuria Railway 1939 |

| | | |
|---|---|---|
| SR 42 | *Rh. formosaensis* Nakazawa 1913 strain No. 1 | Manchuria Railway 1939 |
| SR 43 | *Rh. formosaensis* Nakazawa 1913 var. *chlamydosporus* | Manchuria Railway 1939 |
| SR 44 | *Rh. nodosus* Namyslowski 1906 | A. Niethammer, Prague 1942 |
| SR 45 | *Rh. salebrosus* Yamazaki | A. Niethammer, Prague 1942 |
| SR 46 | *Rh. oryzae* Went a. Pr. Geerl. 1895 strain No. 1 | A. Niethammer, Prague 1942 |
| SR 47 | *Rh. oryzae* Went a. Pr. Geerl. 1895 strain No. 2 | A. Niethammer, Prague 1942 |
| SR 48 | *Rh. oryzae* Went a. Pr. Geerl. 1895 strain No. 3 | A. Niethammer, Prague 1942 |
| SR 49 | *Rh. oryzae* Went a. Pr. Geerl. 1895 strain No. 4 | A. Niethammer, Prague 1942 |
| SR 50 | *Rh. arrhizus* Fischer 1892, strain No. 1 | Zycha, Hannover Münden 1939 |
| SR 51 | *Rh. arrhizus* Fischer 1892, strain No. 2 | Unknown |
| SR 52 | *Rh. arrhizus* Fischer 1892, strain No. 3 | Unknown |
| SR 53 | *Rh. arrhizus* Fischer 1892, strain No. 4 | Zycha, Hannover Münden 1939 |
| SR 54 | *Rh. arrhizus* Fischer 1892, strain No. 5 | C. B. S., Baarn, Kochler |
| SR 55 | *Rh. arrhizus* Fischer 1892, strain No. 6 | A. Niethammer, Prague 1942 |
| SR 56 | *Rh.* species Kocková | isol. fr. soil of Stupčice 1943 |
| SR 57 | *Rh.* species Kocková | isol. fr. soil of Stupčice 1943 |
| SR 58 | *Rh.* species Kocková | isol. fr. soil of Stupčice 1943 |
| SR 59 | *Rh. nigricans* Ehrenberg 1818 strain No. 5 | A. Niethammer, Prague 1942 |
| SR 60 | *Rh. nigricans* Ehrenberg 1818 strain No. 6 | A. Kocková, soil in Prague 1943 |
| SR 61 | *Rh. nigricans* Ehrenberg 1818 strain No. 7 | Lembke, Kiel 1939 |
| SR 62 | *Rh. nigricans* Ehrenberg 1818 strain No. 8 | Botan. Institute Munich, 1941 |
| SR 63 | *Rh. nigricans* Ehrenberg 1818 strain No. 9 | Gärungsinstitut Berlin 1942 |
| SR 64 | *Rh. usamii* Hanzawa 1912 | Manchuria Railway 1939 |
| SR 65 | *Rh.* species Kocková | isol. fr. soil of Prague 1943 |
| SR 66 | *Rh. japonicus* var. *amylomyces* patent | Manchuria Railway 1939 |
| SR 67 | *Rh. nigricans* Ehrenberg 1818 strain No. 10 | Unknown |
| SR 68 | *Rh. formosaensis* Nakazawa 1913 strain No. 2 | Manchuria Railway 1939 |
| SR 70 | *Rh. liquefaciens* Yamazaki | C. B. S., Baarn 1943 |
| SR 72 | *Rh. kasanensis* Hansawa 1912 | C. B. S., Baarn 1943 |
| SR 73 | *Rh. nigricans* Ehrenberg 1818 strain No. 11 | A. Niethammer, Prague 1943 |
| SR 74 | *Rh. delemar* (Boid.) Wehm. and Hanz. 1912, strain No. 5 | A. Niethammer, Prague 1943 |
| SR 75 | *Rh.* species Takeda, strain Peka 1 | C. B. S., Baarn 1943 |
| SR 76 | *Rh. oligosporus* Saito 1905 | C. B. S., Baarn 1943 |
| SR 77 | *Rh. barnensis* Takeda | C. B. S., Baarn 1943 |
| SR 78 | *Rh. maydis* Bruderlein 1917 | C. B. S., Baarn 1943 |
| SR 79 | *Rh. javanicus* Takeda | C. B. S., Baarn 1943 |
| SR 80 | *Rh. batatas* Nakazawa | C. B. S., Baarn 1943 |
| SR 81 | *Rh. achlamydosporus* Takeda | C. B. S., Baarn 1943 |
| SR 82 | *Rh. cohnii* Berlese and de Toni 1888 | C. B. S., Baarn 1943 |
| SR 83 | *Rh. microsporus* van Tieghem 1875 | C. B. S., Baarn 1943 |
| SR 84 | *Rh. fusiformis* Dawson and Povah | C. B. S., Baarn 1943 |
| SR 85 | *Rh. sontii* Red. and Subrahm. | C. B. S., Baarn 1943 |
| SR 86 | *Rh. semarangensis* Takeda | C. B. S., Baarn 1943 |
| SR 87 | *Rh.* species Kocková | isol. fr. soil in Prague 1943 |
| SR 88 | *Rh.* species Kocková | isol. fr. sputum, Prague SZÚ, 1944 |
| SR 89 | *Rh.* species Kocková | isol. fr. sputum, Prague SZÚ, 1944 |
| SR 91 | *Rh.* species Liška | SZÚ, Prague 1944 |
| SR 92 | *Rh.* species Liška | SZÚ, Prague 1944 |

## B. Substrates:

1. Worth agar: Unhopped brewery worth diluted to 8% w/v extr., pH adjusted to 7 by 10% $Na_2CO_3$, 1,5% agar.

2. Bread agar: The bread was boilled, pressed through a sieve, diluted by the brewery worth described above (pH not adjusted), 2% sterile chalk and 1,5% agar added. pH of the medium before the addition of chalk 5,5.

3. Blickfeld agar: 1% peptone Witte, 1% glucose, 1% lactose, 0,5% chalk, 5% yeast extract, 1,5% agar.

## C. Preparation of the cultures:

The agar mediums were poured on Petri dishes of 9 cm. diameter. On the hardened surfaces of the agar diluted suspensions of spores in physiological saline were inoculated by a bacteriological loop. The cultures were incubated at 28° C and they were observed after the fourth day of incubation.

## D. Evaluation of results:

By a micrometer the length of sporangiophors from rhizoid to the sporangium, their width, the length and width of the sporangia and the length of the spores have been measured. Because the sporulation of some strains, especially of Blickfeld agar, on the fourth day of cultivation was very weak and with other strains the measuring of spores was very difficult, an uneven number of measured dimensions was obtained. For the evaluation of measurements the fewest obtained number of measurings was considered and in those cases, where more measurements were taken the same number of the first cases were considered. For this reason the obtained results were not evaluated by the usual biometrical method, but by the analysis of variation, by which it was possible to evaluate the significant differences between strains and the influence of different media. The correlative dependence between the measured values was also established. Beside the measuring of dimensions for every strain the optical temperature was found and for some of the strains the utilisation of nitrogen containing substances was studied.

# Experimental part

## A. Length of sporangiophores

On the three above mentioned nutritional media 71 strains have been tested. For every strain and every medium the sum of five values has been used for statistical evaluation.

| Variability | N | S squares | Square average | F | $F_{5\%}$ | $F_{1\%}$ |
|---|---|---|---|---|---|---|
| Between the strains | 70 | 873.396,502 | 12.478,520 | 169 | 1.37 | 1.56 |
| Between the media | 2 | 7.472,000 | 3.736,000 | 50.7 | 3.06 | 4.75 |
| Residual mistake | 140 | 10.306,700 | 73,619 | | | |
| Total variability | 212 | 891.275,202 | | | | |

As the differences between the media are significant, the composition of the nutritional media has a significant influence on the length of sporangiophores. From the results of the F-test we can judge that there is a significant difference between the length of sporangiophores of different strains.

B. Width of sporangiophores

On the three nutritional media 67 strains have been tested and for every strain the sum of five values has been used for statistical evaluation.

| Variability | N | S squares | Square average | F | $F_{5\%}$ | $F_{1\%}$ |
|---|---|---|---|---|---|---|
| Between the strains | 66 | 55,560.9 | 841.8 | 19.22 | 1.39 | 1.59 |
| Between the media | 2 | 133.9 | 66.9 | 1.35 | 3.07 | 4.78 |
| Residual mistake | 132 | 6,540.8 | 49.5 | | | |
| Total variability | 200 | 62,235.6 | 311.2 | | | |

It was established that the differences between the composition of the nutritional media are not significant, but that there are significant differences between the width of sporangiophores in different strains.

C. Co-efficient of correlation between the length and width of sporangiophores

$\overline{w} = 0.9385 \qquad \sigma_w = 2.5858$

$\overline{v} = 1.4154 \qquad \sigma_v = 3.1275$

$$r_{xy} = \frac{S(w_i v_k) - N\overline{w}\overline{v}}{N\sigma_w\sigma_v} = \frac{465.654}{525.662} = 0.886$$

The calculated co-efficient of correlation was r = 0,886. Between the length and width of the sporangiophores there is a very close relation. From the results summed up in paragraph A, B and C it is possible to draw these conclusions: The length of the sporangiophores is determinatively characteristic for every strain. The length of the sporangiophores is influenced by the composition of the nutritional media as the medium has an effect on the physiological state of the mold. The width of the sporangiophores is not characteristical for the studied strains as the width changes according to the length of the sporangiophors. At the identification of a species of the genus *Rhizopus* according to the dimensions of the sporangiophores only their length is to be considered as determinative and even here one has to consider the influence of the composition of the nutritive medium.

D. Length of sporangia

On three nutritional media 72 strains have been tested and for every strain the sum of five values has been used for statistical evaluation.

| Variability | N | S squares | Square average | F | $F_{5\%}$ | $F_{1\%}$ |
|---|---|---|---|---|---|---|
| Between the strains | 71 | 3.300,589.5 | 46,487.1 | 3.28 | 1.37 | 1.56 |
| Between the media | 2 | 54,133.6 | 27,066.8 | 1.91 | 3.06 | 4·75 |
| Residual mistake | 142 | 2.012,562.6 | 14,172.9 | | | |
| Total variability | 215 | 5.367,285.7 | | | | |

As the calculated F values are inside the limits of significance one can consider that a direct influence of the composition of the nutritional media on the length of sporangia is absent. At the determination of the significance of the differences between different strains the value F was calculated as higher than the value F found in statistical tables for the 5 and 1% limits of probability. A significant difference of the length of the sporangia of different strains was hereby proved.

E. Width of sporangia

On the three media 72 strains have been tested and for every strains the sum of five values has been used for statistical evaluation.

| Variability | N | S squares | Square average | F | $F_{5\%}$ | $F_{1\%}$ |
|---|---|---|---|---|---|---|
| Between the strains | 71 | 3.287,549.0 | 46,303.51 | 2.71 | 1.37 | 1.56 |
| Between the media | 2 | 46,785.96 | 23,392.98 | 1.37 | 3.06 | 4.75 |
| Residual mistake | 142 | 2.423,978.84 | 17,070.27 | | | |
| Total variability | 215 | 5.758,313.80 | | | | |

The differences between nutritional media are not significant, the media do not have a significant influence on the width of the sporangia.

F. Co-efficient of correlation between the length and width of sporangia

$$\bar{w} = 0.3286 \qquad \sigma_w = 3.29$$

$$\bar{v} = 0,3714 \qquad \sigma_v = 3,27$$

$$r_{xy} = \frac{S(w_i v_k) - N\bar{v}\bar{w}}{N\sigma_w\sigma_v} = \frac{736.4571}{743.0810} = 0.978$$

The calculated co-efficient of correlation was r = 0.978. Between the length and width of the sporangia a very close, one could say a nearly functional, correlation was found. For the identification of different strains either length or the width of the sporangia can be used.

G. Length of spores

On the three nutritional media 69 strains have been tested and for every strain and medium the sum of ten values has been used for statistical evaluation.

| Variability | N | S squares | Square average | F | $F_{5\%}$ | $F_{1\%}$ |
|---|---|---|---|---|---|---|
| Between the strains | 68 | 56,481.12 | 962.96 | 14.80 | 1.37 | 1.57 |
| Between the media | 2 | 240.71 | 120.36 | 1.85 | 3.06 | 4.75 |
| Residual mistake | 136 | 8,844.97 | 65.04 | | | |
| Total variability | 206 | 74,566.80 | | | | |

As the calculated F values are inside the limits of significance, we consider that a direct influence of the composition of the nutritional media on the length of the spores is absent. In the determination of the significance of the differences between the different strains the value for F was calculated as higher than the value F found in statistical tables for the 5 and 1% limits of probability. A significant difference between the length of spores of different strains was hereby proved.

### H. Correlation between the length of spores and length of sporangia

$$\bar{w} = 0.1940 \qquad \sigma_w = 3.12 \qquad \bar{v} = 0.8209 \qquad \sigma_v = 2.71$$

$$r_{xy} = \frac{S(w_i v_k) - N\bar{w}\bar{v}}{N\sigma_w\sigma_v} = \frac{198.670}{566.4984} = 0.351$$

Verification of the significance by the t-test:

$$t = \frac{r_{xy}\sqrt{r-2}}{\sqrt{1-r_{xy}^2}} = 3.1 \qquad t_{60,5\%} = 2.0$$

The correlation is existing.

The correlation between the length of the spores and the length of the sporangia is rather small, but after the verification by the t-test it is evident, that a certain correlation does exist.

### J. Optimal growth temperature

By studying the optimal growth temperature of the strains of the genus Rhizopus we have found that they prefer temperatures which prevailed in the natural environment of the mold, but by long subcultivation in other temperatures the organisms get accustomed to these new conditions. Only the freshly isolated strains, for instance the strains SR 58 and SR 59 have preserved the property which Zycha uses as a distinguishing mark: Strains which according to their characteristics correspond to the description of *Rhizopus nigricans* had a very low optimal temperature of 22° C and at a temperature of 37° C did grow very slowly. But many strains which according to their original description had an optimal temperature 37° C showed the most abundant growth at temperature near 30° C. Accordingly the optimal growth temperature can be used as an identification mark only for freshly isolated cultures from natural sources and this temperature is not to be used for the reidentification of strains.

### K. Formation of gemmae

The formation of gemmae was always dependent on the cultivating conditions and was induced on deficient media with less nutritive components as is the case with Blickfelds agar.

### Summary

1) It is not possible to differenciate various strains of the genus *Rhizopus* according to the length of their sporangiophores unless the composition of the nutritive medium and the growth conditions are accounted for. Other morphological marks, for instance the sizes of sporangia and spores are not directly influenced by the composition of the medium, but rather by the development of the whole fruiting body. The relation of the dimension of various organs, e. g. the width and length of the sporangia, the length of spores and sporangia,

the width and length of sporangiophores prove this fact. Between various strains there are differences in the length and width of the sporangia, sporangiophores and spores.

2) The mentioned distinguishing marks can be supplemented also by some other factors, for instance by the relation of the width and length of sporangiophores. In doing so it is possible to divide the members of the genus *Rhizopus* in three groups:

a) Big strains, where the length of the sporangiophores is 80—100 times greater than the width of these organs (e. g. *Rh. oryzae* and *Rh. nigricans*).
b) Medium sized strains, where the length of the sporangiophores is 60—80 times greater than their width (e. g. *Rh. circinans*).
c) Subtle strains, where the sporangiophores are maximally 50 times longer than their width (e. g. *Rh. arrhizus* and *Rh. oligosporus*).

3) According to our experiences *Rh. microsporus* is not to be considered as an independent species as we have found spores of small dimensions in some strains, while by their properties they belonged to the big species.

4) Swollen sporangiophores are not a specifical mark of the species *Rh. arrhizus* as they can be found also on different species, for instance on the species. *Rh. oryzae*. We have often found characteristically widened ends of sporangiophores in the species *Rh. oryzae* (Fig. 2). One has to distinguish between the "joint-like" swellings, which most often occur in the species *Rh. arrhizus* and between the swellings located on the ends of the sporangiophores of the species *Rh. oryzae*. For the distinguishing between these species other morphological marks and the ratio of the length and width of the sporangiophores are to be used.

5) Although we have worked with 77 identified strains originating from various locations of Europe and Asia and with strains isolated by us from soil and parasitic sources, we have never found the species corresponding with description of *Rhizopus echinatus* van Tieghem 1876. This species is also not described in the papers of Dyr (1937—38, 1939, 1941), Niethammer (1933), Krehl-Nieffer (1951) and not even in the last work of Bernát (1954). Zycha alone admits that this species is uncertain. We suppose that in the original description probably a different species of the family of Mucorineae (perhaps *Cunninghamella echinulata*) was mistaken for this species.

6) According to these marks we have divided the species of the genus *Rhizopus* in groups by help of this key:

A. Big and medium sized strains with strong and long sporangiophores. Length- width ratio 1 : 80—100:
  a) Sporangia overhanging ........*Rh. circinans*
  Sporangia on upstanding sporangiophores:
    b) Spores small 7—10 $\mu$ long, optimal temperature 30° C ........*Rh. oryzae*
    Spores big, 10—20 $\mu$, optimal temperature 22° C ........*Rh. nigricans*
B. Smaller subtle strains, sporangiophore shorter. Length. width ratio 1 : 50 or less.
  a) Spores small, 5—9 $\mu$ long, sporangiophores with joint-like swellings ........*Rh. arrhizus*
  Spores big, 7—12 $\mu$ long, sporangiophores without swellings, but sometimes with widened ends ..................*Rh. oligosporus*.

*Rhizopus circinans* (Fig. 4) is a big species with long, strong sporangiophores with a width-length ratio of 1 : 60—80. The sporangia are big and heavy so, that the ends of the sporangiophores are overhanging.

*Rhizopus oryzae* (Fig. 2) is a big species with strong sporangiophores which are sometimes widened on the ends. They are always upstanding so that the sporangia never hang down. The width-length ratio of the sporangiophores is 1 : 80—100. Spores are relatively small, 7—11 μ long. Most abundant growth observad at higher temperature of about 30 °C.

*Rhizopus nigricans* (Fig. 6) is a big species with long, strong sporangiophores, the sporangia of which are never overhanging. The width-length ratio of the sporangiophores is 1 : 80—100.Spores are big, 10—20 μ long. The optimal growth temperature is lower, about 22 °C.

*Rhizopus arrhizus* (Fig. 1) is a subtle species with shorter sporangiophores. The width-length ratio is about 1 : 50. On the sporangiophores jointlike swellings are formed. In the swellings branching of the sporangiophores is often observed. Spores are small, about 5—9 μ long, sporangiophores are often observed.

*Rhizopus oligosporus* (Fig. 5) is also a subtle species with shorter sporangiosphores with a width-length ratio about 1 : 50. The spores are bigger, 7—12 μ long. This species never forms swellings in the middle of the sporangiophores. Widened ends of the sporangiophores are sometimes observed.

7) According to these characteristics of the various species it is possible to arrange the studied strains followingly:

1. *Rhizopus circinans*:
   SR 34, SR 35, SR 38, SR 39.
2. *Rhizopus oryzae*:
   SR 2, SR 4, SR 5, SR 7, SR 33, SR 45, SR 46, SR 47, SR 48, SR 49.

c) *Rhizopus nigricans*:
   SR 37, SR 50, SR 58, SR 59, SR 61, SR 65, SR 73, SR 79, SR 88, SR 89.

d) *Rhizopus arrhizus*:
   SR 6, SR 8, SR 11, SR 18, SR 20, SR 44, SR 51, SR 52, SR 53, SR 54, SR 55, SR 56, SR 57, SR 63, SR 68, SR 87.

e) *Rhizopus oligosporus*:
   SR 9, SR 10, SR 12, SR 13, SR 26, SR 30, SR 31, SR 32, SR 40, SR 42, SR 43, SR 60, SR 62, SR 64, SR 66, SR 67, SR 70, SR 72, SR 74, SR 75, SR 76, SR 78, SR 77, SR 80, SR 81, SR 82, SR 83, SR 84, SR 85, SR 86, SR 91, SR 92.

*Rhizopus delemar* SR 21, *Rhizopus nigricans* Kaki SR 22, *Rhizopus* species Nill SR 23, *Rhizopus* species Nill SR 24 and *Rhizopus* species Tanaka SR 27 have been found not to be members of the genus *Rhizopus*, but they belonged to the genus *Absidia*.

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E x p l a n a t i o n s t o t h e t a b. X. a n d XI.

1. *Rhizopus arrhizus.*
2. Collumela of the *Rhizopus oryzae.*
3. Rhizoid of the *Rhizopus oligosporus.*
4. *Rhizopus circinans.*
5. *Rhizopus oligosporus.*
6. *Rhizopus nigricans.*

Table 1. *Rhizopus circinans*

| Number | Width Spf | Length Spf | Ratio W : L | Length of spores | Descr. of spores | Length Spg | Width Spg | Ratio W : L | Opt. temp. | Color Spf | Mycelial mad | Ratio spores : Spg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR 34 | 16.9 | 1,235 | 1 : 72 | 8.7 | oval smooth | 108 | 108 | 1 : 1 | 28° C | dark brown | high | 1 : 12 |
| SR 35 | 16.0 | 1,190 | 1 : 74 | 7.4 | oval smooth | 98 | 92 | 1 : 1 | 28° C | dark brown | high | 1 : 14 |
| SR 38 | 15.2 | 1,291 | 1 : 84 | 8.8 | oval smooth | 145 | 135 | 1 : 1 | 28° C | dark brown | high | 1 : 16 |
| SR 39 | 18.3 | 1,456 | 1 : 81 | 9.5 | oval smooth | 134 | 131 | 1 : 1 | 28° C | dark brown | high | 1 : 16 |

Table 2. *Rhizopus oryzae*

| Number | Width Spf | Length Spf | Rato W : L | Length of spores | Descr. of spores | Length Spg | Width Spg | Ratio W : L | Opt. temp. | Color Spf | Mycelial mad | Ratio spores : Spg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR 2 | 12,8 | 1.062 | 1 : 83 | 9,1 | oval streaked | 111 | 105 | 1 : 1 | 28° C | brown | high brown. black | 1 : 12 |
| SR 4 | 17,0 | 1.584 | 1 : 88 | 10,0 | oval streaked | 126 | 123 | 1 : 1 | 28° C | brown gray | high brown. black | 1 : 12 |
| SR 5 | 12,2 | 749 | 1 : 62 | 7,4 | oval dotted | 105 | 103 | 1 : 1 | 30° C | brown gray | high brown. black | 1 : 15 |
| SR 7 | 11,6 | 801 | 1 : 60 | 8,4 | oval streaked | 123 | 106 | 1 : 1,2 | 30° C | brown gray | high brown. black | 1 : 12 |
| SR 33 | 12,0 | 1.089 | 1 : 90 | 10,5 | oval streaked | 113 | 112 | 1 : 1 | 37° C | brown | high brown. black | 1 : 11 |
| SR 45 | 20,2 | 1.352 | 1 : 67 | 7,1 | oval streaked | 104 | 99 | 1 : 1 | 30° C | grayish black | high black | 1 : 15 |
| SR 46 | 11,6 | 1.038 | 1 : 90 | 6,9 | oval streaked | 101 | 100 | 1 : 1 | 37° C | brown | high black | 1 : 14 |
| SR 47 | 12,4 | 1.140 | 1 : 92 | 7,6 | oval streaked | 102 | 100 | 1 : 1 | 37° C | brown | high black | 1 : 13 |
| SR 48 | 12,4 | 1.128 | 1 : 91 | 7,0 | oval smooth | 102 | 97 | 1 : 1 | 37° C | brown | high black | 1 : 14 |
| SR 49 | 10,9 | 1.035 | 1 : 95 | 7,3 | oval smooth | 110 | 101 | 1 : 1 | 37° C | brown | high black | 1 : 15 |

Table 3. *Rhizopus nigricans*

| Number | Width Spf | Length Spf | Ratio W : L | Length of spores | Descr. of spores | Length Spg | Width Spg | Ratio W : L | Opt. temp. | Color Spf | Mycelial mad | Ratio spores : Spg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR 37 | 13.4 | 1,015 | 1 : 82 | 11.3 | oval streaked | 158 | 156 | 1 : 1 | 22° C | brown | black high | 1 : 14 |
| SR 50 | 21.8 | 1,815 | 1 : 82 | 10.1 | oval streaked | 97 | 86 | 1 : 1 | 28° C | brown. black | black high | 1 : 9,7 |
| SR 58 | 17.4 | 1,459 | 1 : 85 | 13.1 | oval streaked | 111 | 111 | 1 : 1 | 22° C | brown. black | black high | 1 : 8,5 |
| SR 59 | 19.3 | 1,861 | 1 : 97 | 11.3 | oval streaked | 141 | 136 | 1 : 1 | 22° C | brown. black | black high | 1 : 12 |
| SR 61 | 20.6 | 1,796 | 1 : 89 | 11.5 | oval streaked | 126 | 121 | 1 : 1 | 22° C | brown. gray | black high | 1 : 11 |
| SR 65 | 26.2 | 2,462 | 1 : 94 | 11.4 | oval streaked | 164 | 162 | 1 : 1 | 22° C | brown. black | black high | 1 : 14 |
| SR 73 | 17.8 | 1,533 | 1 : 85 | 10.5 | oval streaked | 86 | 85 | 1 : 1 | 28° C | brown | black high | 1 : 8,6 |
| SR 79 | 25.3 | 1,178 | 1 : 47 | 12.7 | oval streaked | 146 | 143 | 1 : 1 | 28° C | brown | black high | 1 : 11 |
| SR 88 | 20.4 | 1,747 | 1 : 87 | 11.8 | oval streaked | 122 | 121 | 1 : 1 | 22° C | brown | black high | 1 : 10 |
| SR 89 | 19.0 | 1,535 | 1 : 80 | 10.8 | oval streaked | 116 | 115 | 1 : 1 | 28° C | brown | black high | 1 : 11 |

Table 4. *Rhizopus arrhizus*

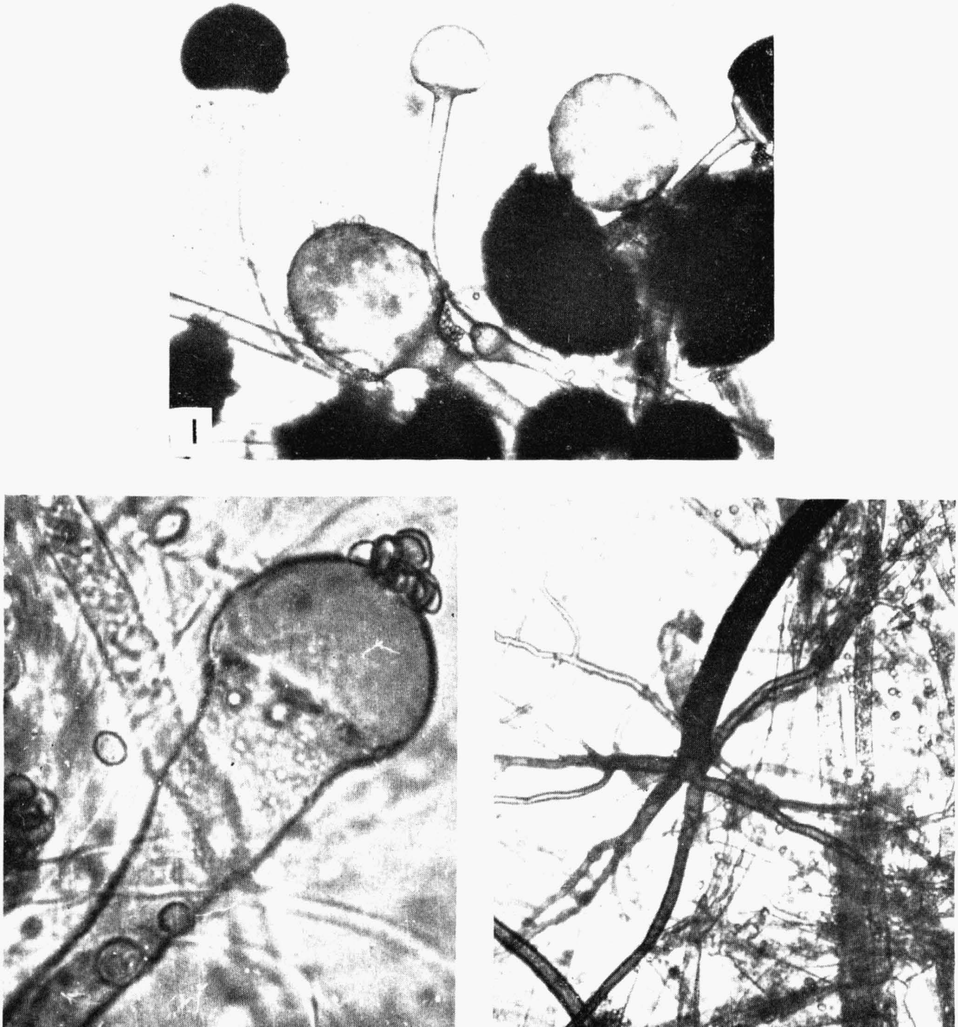
| Number | Width Spf | Length Spf | Rato W : L | Length of spores | Descr. of spores | Length Spg | Width Spg | Ratio W : L | Opt. temp. | Color Spf | Mycelial mad | Ratio spores : Spg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR 6 | 12.3 | 609 | 1 : 50 | 6.4 | oval smooth | 87 | 87 | 1 : 1 | 28° C | brown swell. | dark gray medium | 1 : 14 |
| SR 8 | 13.1 | 566 | 1 : 44 | 8.0 | oval streaked | 112 | 106 | 1 : 1 | 28° C | brown swell. | dark gray medium | 1 : 13 |
| SR 11 | 14( ?) | 703 | 1 : 36 | 8.1 | oval streaked | 139 | 133 | 1 : 1 | 28° C | brown swell. | dark gray medium | 1 : 16 |
| SR 18 | 11.2 | 638 | 1 : 58 | 5.6 | oval streaked | 114 | 108 | 1 : 1 | 28° C | brown swell. | dark gray medium | 1 : 18 |
| SR 20 | 12.0 | 700 | 1 : 58 | 6.3 | oval smooth | 102 | 96 | 1 : 1 | 28° C | brown swell. | dark gray medium | 1 : 16 |
| SR 44 | 11.5 | 600 | 1 : 54 | 6.3 | oval streaked | 104 | 98 | 1 : 1 | 28° C | brown swell. | dark gray medium | 1 : 16 |
| SR 51 | 11.9 | 603 | 1 : 50 | 6.5 | oval streaked | 96 | 94 | 1 : 1 | 28° C | brown swell. | dark gray medium | 1 : 15 |
| SR 52 | 11.0 | 699 | 1 : 63 | 9.0 | oval streaked | 68 | 68 | 1 : 1 | 37° C | brown swell. | dark gray medium | 1 : 7.5 |
| SR 53 | 10.9 | 648 | 1 : 63 | 7.5 | oval streaked | 93 | 84 | 1 :1 | 37° C | brown swell. | dark gray medium | 1 : 11 |
| SR 54 | 10.1 | 560 | 1 : 56 | 7.8 | oval streaked | 77 | 77 | 1 : 1 | 37° C | brown swell. | dark gray medium | 1 : 9.6 |
| SR 55 | 11.3 | 625 | 1 : 56 | 9.3 | oval streaked | 95 | 93 | 1 : 1 | 37° C | brown swell. | white | 1 : 10.5 |
| SR 56 | 10.9 | 656 | 1 : 58 | 8.3 | oval smooth | 77 | 75 | 1 : 1 | 28° C | brown swell. | white | 1 : 9.6 |
| SR 57 | 10.7 | 670 | 1 : 60 | 8.2 | oval streaked | 72 | 75 | 1 : 1 | 28° C | brown swell. | white | 1 : 9 |
| SR 63 | 13.1 | 471 | 1 : 36 | 6.5 | oval streaked | 81 | 75 | 1 : 1 | 28° C | dark swell. | dark | 1 : 12 |
| SR 68 | 13.5 | 907 | 1 : 66 | 8.2 | oval streaked | 97 | 94 | 1 : 1 | 28° C | brown swell. | dark | 1 : 11 |
| SR 87 | 13.3 | 626 | 1 : 48 | 6.8 | oval streaked | 94 | 94 | 1 : 1 | 37° C | brown swell. | brown | 1 : 13 |

Table 5. *Rhizopus oligosporus*

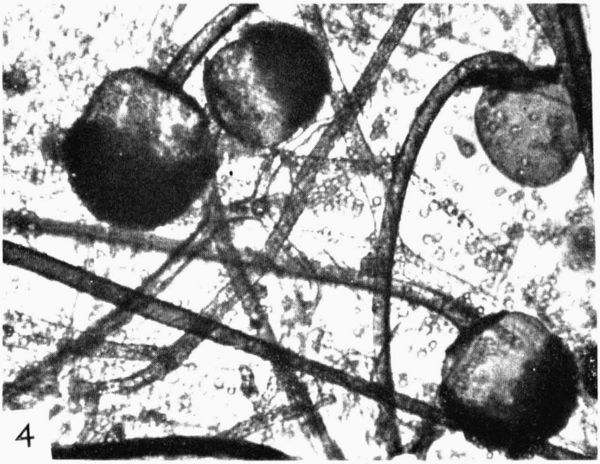
| Number | Width Spf | Length Spf | Ratio W : L | Length of spores | Descr. of spores | Length Spg | Width Spg | Ratio W : L | Opt. temp. | Color Spf | Mycelial mad | Ratio spores : Spg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR 9 | 13.1 | 524 | 1 : 40 | 6.9 | oval streaked | 93 | 88 | 1 : 1 | 28° C | brown | bluish gray medium | 1 : 12 |
| SR 10 | 11.4 | 600 | 1 : 54 | 6.9 | oval streaked | 93 | 92 | 1 : 1 | 28° C | brown | bluish gray medium | 1 : 13 |
| SR 12 | 14.0 | 662 | 1 : 47 | 7.2 | oval smooth | 93 | 93 | 1 : 1 | 28° C | brown | bluish gray medium | 1 : 13 |
| SR 13 | 12.9 | 670 | 1 : 51 | 7.6 | oval smooth | 111 | 110 | 1 : 1 | 28° C | brown | bluish gray medium | 1 : 16 |
| SR 26 | 12.0 | 660 | 1 : 55 | 8.6 | streaked oval | 108 | 103 | 1 : 1 | 37° C | brown | bluish gray medium | 1 : 14 |
| SR 30 | 11.7 | 780 | 1 : 65 | 8.9 | oval streaked | 90 | 89 | 1 : 1 | 28° C | brown | bluish gray medium | 1 : 10 |
| SR 31 | 13.3 | 829 | 1 : 73 | 8.2 | oval smooth | 100 | 100 | 1 : 1 | 28° C | brown | bluish gray medium | 1 : 12 |
| SR 32 | 12.3 | 623 | 1 : 52 | 7.0 | oval smooth | 100 | 98 | 1 : 1 | 37° C | brown | bluish gray medium | 1 : 14 |
| SR 40 | 14.3 | 552 | 1 : 40 | 8.8 | oval smooth | 86 | 84 | 1 : 1 | 30° C | brown | bluish gray medium | 1 : 10 |
| SR 42 | 10.9 | 508 | 1 : 46 | 8.2 | oval smooth | 73 | 71 | 1 : 1 | 30° C | brown | bluish gray medium | 1 : 8 |
| SR 43 | 10.8 | 603 | 1 : 55 | 9.1 | sphaer. smooth | 74 | 73 | 1 : 1 | 28° C | brown | bluish gray medium | 1 : 8 |
| SR 60 | 10.2 | 506 | 1 : 50 | 6.3 | oval smooth | 69 | 68 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 10 |
| SR 62 | 14.0 | 700 | 1 : 50 | 6.6 | oval smooth | 92 | 92 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 13 |
| SR 64 | 12.8 | 702 | 1 : 54 | 6.9 | oval smooth | 85 | 83 | 1 : 1 | 28° C | brown | bluish gray higner | 1 : 11 |
| SR 66 | 13.8 | 593 | 1 : 42 | 7.0 | irregul. streaked | 101 | 100 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 14 |
| SR 67 | 12.0 | 506 | 1 : 50 | 6.3 | oval smooth | 69 | 68 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 14 |
| SR 70 | 13.0 | 575 | 1 : 44 | 7.5 | oval smooth | 95 | 93 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 14 |
| SR 72 | 16.2 | 541 | 1 : 34 | 6.8 | sphaer. dotted | 81 | 79 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 11 |

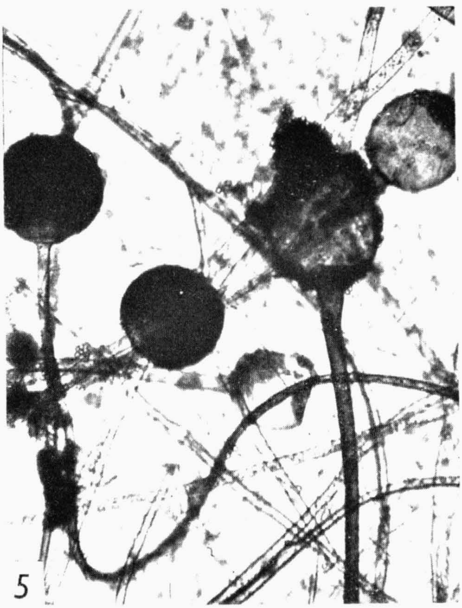
(Continuation of table 5)

| Number | Width Spf | Length Spf | Rato W : L | Length of spores | Descr. of spores | Length Spg | Width Spg | Ratio W : L | Opt. temp. | Color Spf | Mycelial mad | Ratio spores : Spg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR 74 | 12.3 | 592 | 1 : 50 | 6.7 | oval smooth | 93 | 86 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 12 |
| SR 75 | 11.3 | 660 | 1 : 60 | 9.0 | oval smooth | 93 | 88 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 10 |
| SR 76 | 12.4 | 592 | 1 : 50 | 9.2 | oval smooth | 71 | 69 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 8 |
| SR 77 | 11.8 | 589 | 1 : 50 | 10.7 | oval smooth | 83 | 83 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 8 |
| SR 80 | 13.9 | 740 | 1 : 53 | 10.7 | oval smooth | 101 | 99 | 1 : 1 | 28° C | brown | bluish gray higher | 1 : 10 |
| SR 81 | 13.5 | 637 | 1 : 47 | 7.2 | oval streaked | 99 | 95 | 1 : 1 | 28° C | brown-ish | dark bluish gray | 1 : 8 |
| SR 82 | 12.9 | 638 | 1 : 49 | 9.7 | oval streaked | 85 | 78 | 1 : 1 | 37° C | brown-ish | dark bluish gray | 1 : 8 |
| SR 83 | 13.4 | 729 | 1 : 56 | 8.8 | oval streaked | 102 | 97 | 1 : 1 | 28° C | brown-ish | dark bluish gray | 1 : 11 |
| SR 84 | 14.4 | 877 | 1 : 62 | 8.8 | oval streaked | 124 | 122 | 1 : 1 | 28° C | brown-ish | dark bluish gray | 1 : 15 |
| SR 85 | 14.6 | 780 | 1 : 55 | 9.0 | oval streaked | 99 | 97 | 1 : 1 | 28° C | brown-ish | dark bluish gray | 1 : 10 |
| SR 86 | 14.6 | 712 | 1 : 48 | 9.0 | oval streaked | 102 | 100 | 1 : 1 | 28° C | brown-ish | dark bluish gray | 1 : 11 |
| SR 91 | 12.6 | 638 | 1 : 49 | 10.7 | oval streaked | 70 | 70 | 1 : 1 | 28° C | brown-ish | dark bluish gray | 1 : 7 |
| SR 92 | 12.1 | 591 | 1 : 50 | 10.2 | oval streaked | 94 | 94 | 1 : 1 | 28° C | brown-ish | dark bluish gray | 1 : 7 |




A. Kocková-Kratochvílová and V. Palkoska: A. Taxonomic Study of the Genus *Rhizopus* Ehrenberg 1820.





A. K o c k o v á - K r a t o c h v í l o v á and V. P a l k o s k a : A. Taxonomic Study of the Genus *Rhizopus* E h r e n b e r g 1820.