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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 characteristic 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 than 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 μ 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. Sporangiphors and rhizoids delicate, maximally 1 mm high,
 optimal growthtemperature 30° C (5)
 Sporangiphors and rhizoids strong, even 2—4 mm. high ... (7)
5. Spores 7—12 μ long....*Rh. oligosporus* Saito 1905
 Synonyma:
Rhizopus delemar (Boidin) Wehmer and Hanzawa 1912
Rhizopus tamari Saito 1907
- Spores 5—7 μ long (6)
6. Sporangiphors longer
 than 150 μ *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 pusillus 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 μ *Rh. cohnii* Berlese and de Toni 1888
 Synonyma:
Rhizopus suinus Nielsen 1929
7. Spores 7—9 μ 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 μ 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 arthocarpì 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:

<i>Rhizopus acidus</i> Yamazaki	<i>Rhizopus chinniang</i> Yamazaki
<i>Rhizopus chungkuoensis</i> Yamazaki	<i>Rhizopus formosaeensis</i> Nakazawa
<i>Rhizopus hangchao</i> Yamazaki	<i>Rhizopus humilis</i> Yamazaki
<i>Rhizopus kasanensis</i> Hanzawa	<i>Rhizopus liquefaciens</i> Yamazaki
<i>Rhizopus niveus</i> Yamazaki	<i>Rhizopus pseudochinensis</i> Yamazaki
<i>Rhizopus salebrosus</i> Yamazaki	<i>Rhizopus shanghaiensis</i> Yamazaki
<i>Rhizopus trubini</i> Hanzawa	<i>Rhizopus usami</i> Hanzawa
<i>Rhizopus apiculatus</i> Mac Alpine	<i>Rhizopus fusiformis</i> Dawson and Povah
<i>Rhizopus pigmaeus</i> Naumov	<i>Rhizopus schizans</i> Mac Alpine
<i>Rhizopus umbellatus</i> Smith	

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

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

$\bar{w} = 0.9385$
 $\bar{v} = 1.4154$

$\sigma_w = 2.5858$
 $\sigma_v = 3.1275$

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

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

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

$$\bar{v} = 0.3714 \qquad \sigma_v = 3.27$$

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

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

$$\begin{array}{ll} \bar{w} = 0.1940 & \sigma_w = 3.12 \\ \bar{v} = 0.8209 & \sigma_v = 2.71 \end{array} \quad r_{xy} = \frac{S(w_i v_i) - N \bar{w} \bar{v}}{N \sigma_w \sigma_v} = \frac{198.670}{566.4984} = 0.351$$

Verification of the significance by the t-test:

$$t = \frac{r_{xy} \sqrt{r-2}}{\sqrt{1-r^2}} = 3.1 \quad t_{60,5\%} = 2.0 \quad \text{The correlation is existing.}$$

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 differentiate 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 sporangio-phores. In doing so it is possible to divide the members of the genus *Rhizopus* in three groups:

- 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*).
- Medium sized strains, where the length of the sporangiophores is 60—80 times greater than their width (e. g. *Rh. circinans*).
- 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 specific 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), Niehammer (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 μ long, optimal temperature 30° C
.....*Rh. oryzae*
Spores big, 10—20 μ , optimal temperature 22° C
.....*Rh. nigricans*
- B. Smaller subtle strains, sporangiophore shorter. Length. width ratio 1 : 50 or less.
- a) Spores small, 5—9 μ long, sporangiophores with joint-like swellings
.....*Rh. arrhizus*
Spores big, 7—12 μ long, sporangiophores without swellings, but sometimes with widened ends*Rh. oligosporus*.

Rhizopus circinans (Fig. 4) is a big species with long, strong sporangio-phores 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 observed 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 sporangiophores 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.
- e) *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* K a k i SR 22, *Rhizopus* species N i l l SR 23, *Rhizopus* species N i l l SR 24 and *Rhizopus* species T a n a k a SR 27 have been found not to be members of the genus *Rhizopus*, but they belonged to the genus *Absidia*.

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Explanations to the tab. X. and 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	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 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	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*

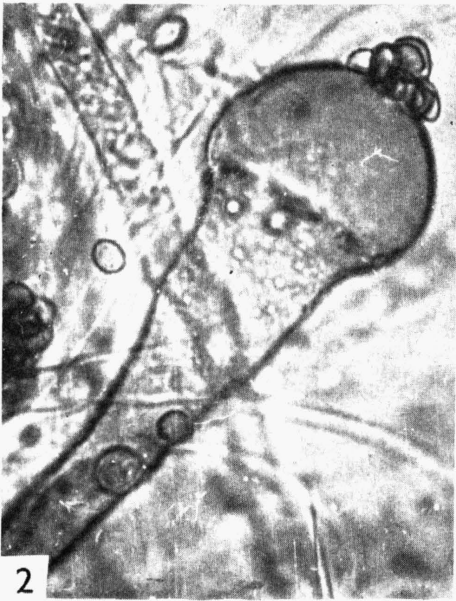
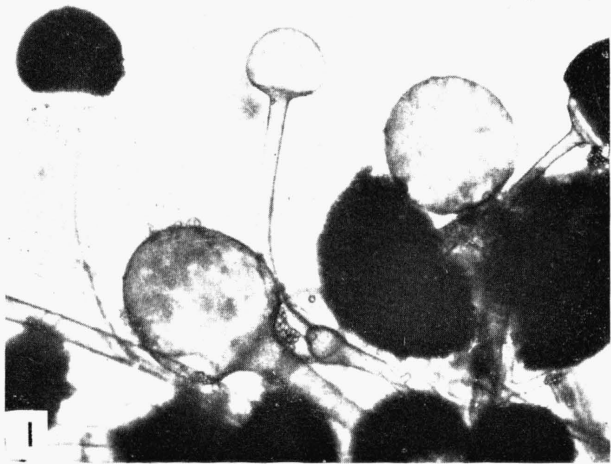
Number	Width Spf	Length Spf	Ratio W : L	Length of spores	Descr. of spores	Length Spg	Width Spg	Ratio W : L	Opt. temp.	Color Spf	Mycelial mat	Ratio spores : Spg
SR 6	12.3	609	1 : 50	6.4	oval smooth	87	87	1 : 1	28° C	brown swell.	dark gray medium	1 : 14
SR 8	13.1	566	1 : 44	8.0	oval streaked	112	106	1 : 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*

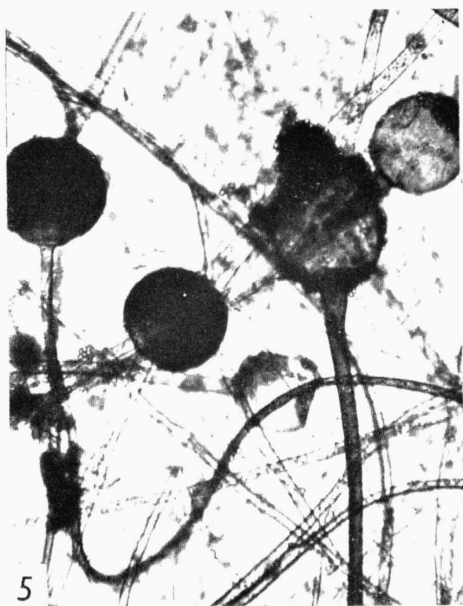
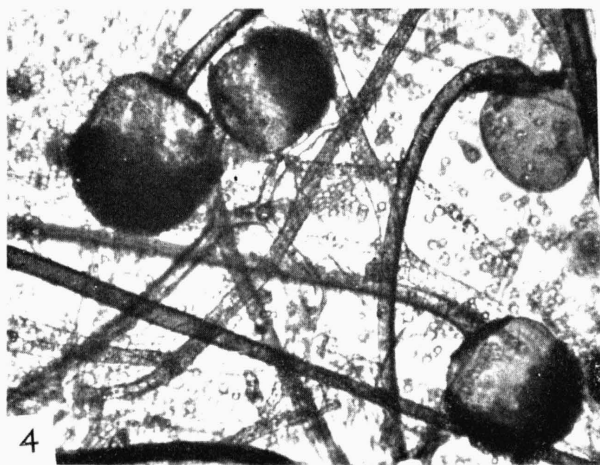
Number	Width Spf	Length Spf	Ratio W : L	Length of spores	Descr. of spores	Length Spg	Width Spg	Ratio W : L	Opt. temp.	Color Spf	Mycelial mat	Ratio spores : Spg
SR 9	13.1	524	1 : 40	6.9	oval streaked	93	88	1 : 1	28° C	brown	bluish gray medium bluish	1 : 12
SR 10	11.4	600	1 : 54	6.9	oval streaked	93	92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

(Continuation of table 5)

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



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



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