On some sporogenous yeasts and their imperfect stages

by

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With 3 figures.

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Introduction

Taxonomy of yeasts is based in the first place on the presence or absence of the ability to produce ascospores. The spore-forming yeasts belong to the ASCOMYCETES, whereas the yeasts lacking this ability have to be placed in the large group of the FUNGI IMPERFECTI. Hence in classificatory practice it is of primary importance to establish whether a yeast strain is able to produce ascospores or not.

Notwithstanding all that, this point of view has not seldom been neglected. Since by far the majority of the pathogenic yeasts are anascosporogenous, it is not rare to find that medical mycologists have omitted the examination of the sporeforming ability of yeast strains isolated by them. And even when this quality has more or less accidentally been established, the result has often been ignored in the determination of the taxonomic position of the yeast.

For this reason we have deemed it necessary, in making a systematic survey of the numerous supposedly anascosporogenous yeast cultures present in the collection of the „Centraalbureau voor Schimmelcultures", to re-examine all these cultures on the possible presence of the ability to form spores.

In total nearly 300 cultures have been investigated, nine of which yielded a positive result.

It seems worth-while to report here on these cases, because this result involves a change in taxonomy of some species, partly well known in medical mycology.

There is, however, still a second reason which made us decide to publish this part of our critical survey of the anascosporogenous yeast species separately. Until now the classification of sporogenous and that of asporogenous yeasts have developed largely along quite independent lines. As far as the pseudomycelium-forming representatives of the latter group (*Mycotoruloideae*) are concerned, we owe of late to LANGERON and his collaborators reliable methods for the determination of valuable morphological characters (mode of attachment of blastospores to pseudomycelium etc.). It now has become tempting to apply the same investigatory methods to sporogenous yeast cultures as well. In doing so, and by taking at the same time the physiological characters into account, it has become possible to furnish satisfactory evidence that indeed various asporogenous yeasts have to be considered as imperfect stages of partly well-known sporogenous species.

The results reported below offer several examples of this point of view. A continuation of this study along the line indicated, *i.e.* a systematic study of the pseudomycelium-forming ability of sporogenous yeast species, might, moreover, in the long run markedly influence the principles to be used in the classification of the sporogenous yeasts.

Experimental results and taxonomic conclusions

In testing the various yeast cultures on their spore-forming ability use has been made of all better-known methods, *viz.* the "block of plaster" method, the inoculation from young malt agar cultures on carrot, on potato, and on GORODKOWA-agar.

Taking into account also the other characteristics of the nine species yielding a positive result, it was possible to establish that six of them did belong to the genus *Saccharomyces*, two to the genus *Hansenula*, and one to the genus *Debaryomyces*.

A. We shall now first discuss the strains belonging to the genus *Saccharomyces*:

I. *Mycocandida pinoyisimilis* (A. Cast.) Red. et Cif. var. *Citelliana* Red. et Cif.

This culture has been described by CIFERRI and REDAELLI in 1935 [1]). It was isolated by CAÑCÒ at Catania from a human lesion of the tonsils and the pharynx. The "Centraalbureau" received this culture in May 1934 from CIFERRI and REDAELLI.

Ascospores were observed by us on a slice of carrot after an incubation -period of one week. Each ascus contains four or less spores. For the greater part the spores are reniform. With exception of this ascospore-formation the morphological characteristics of this culture, as described by CIFERRI and REDAELLI, correspond very well to those observed by us. Slide cultures on peptone-glucose agar [after the technique of RIVALIER and SEYDEL [2])] — a method which has come up of late to study the morphology of yeasts, especially with regard to the formation of pseudomycelium or mycelium with the arrangement of blastospores — showed the same appearance (Fig. 1) as is reproduced on the photo of the Italian investigators (cf. Fig. 15 in the publication of CIFERRI and REDAELLI). However, the results of the fermentation-tests made by these investigators do not correspond very well to our observations. CIFERRI and REDAELLI reported this culture to be able to ferment glucose, fructose and saccharose, whereas we observed fermentation of glucose fructose, mannose, galactose, saccharose and lactose, and not of maltose. But as these authors state that the results of two independently made tests on sugar fermentation were diverging, while we got in all of our tests the same results, we think we may accept the correctness of our own results.

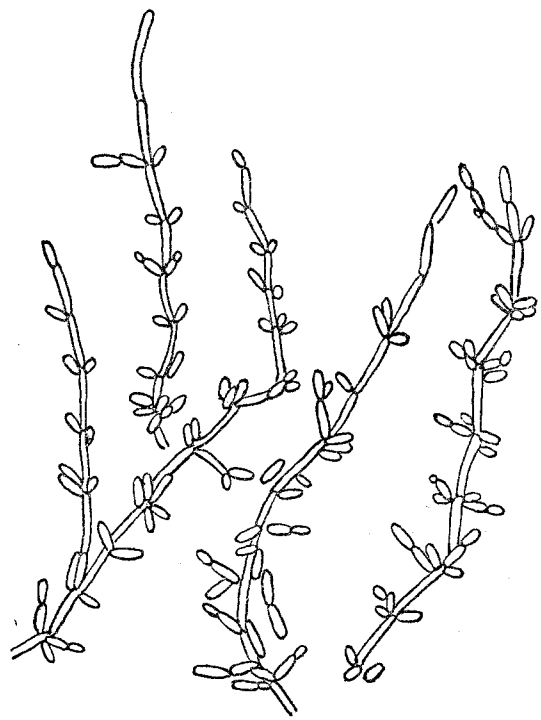

Fig. 1.

As for the systematic place of this organism among the sporogenous yeasts, it evidently belongs to the genus *Saccharomyces* and it is completely identical with *Saccharomyces fragilis* Jörgensen [3]). The latter organism, isolated by the author of the species from kefir, is, at least in Holland, frequently found in buttermilk.

The culture we are dealing with shows a striking resemblance with a culture labelled

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[1]) R. CIFERRI e P. REDAELLI, Archiv f. Mikrobiol., **6**, 9, (1935).

[2]) E. RIVALIER et S. SEYDEL, Compts. rend. Soc. Biol., **40**, 181 (1932); Ann. d. Parasitol. hum. et comp., **10**, 444, (1932).

[3]) Cf. N. M. STELLING-DEKKER, Die Hefesammlung des „Centraalbureau voor Schimmelcultures". Erster Teil, Die sporogenen Hefen. Verh. Kon. Akad. v. Wetensch. **28**, (1931), (p. 148).

Monilia pseudotropicalis A. Cast. received from CASTELLANI in September 1935. This culture, an authentic one, corresponds very well to the description of CASTELLANI [1]), which, however, is based on biochemical characteristics only. As is pointed out of late by several investigators the name *Monilia* has, however, to be disregarded in designating yeasts. We wish to use here the generic name *Candida* sensu BERKHOUT [2]), intending to discuss elsewhere these questions of nomenclature more extensively. BERKHOUT herself did not study this culture, but a great number of other yeastlike "*Monilias*" for which she established the generic name *Candida*.

According to DODGE [3]), BASGAL in 1931 placed *Monilia pseudotropicalis* in the genus *Candida*.

Candida pseudotropicalis (A. Cast.) Basgal does not produce ascospores, but is in all other respects, morphologically as well as physiologically, identical with the culture designated by CIFERRI and REDAELLI as *Mycocandida pinoyisimilis* (A. Cast.) Red. et Cif. var. *Citelliana* Red. et Cif. (= *Saccharomyces fragilis* Jörgensen, cf. p. 29). As the morphology of the sporogenous yeasts has not been studied with the slide culture-method of RIVALIER and SEYDEL, we investigated several strains of *Saccharomyces fragilis* of the collection of the "Centraalbureau" according to this method: all of them with the same results. They produced well developed pseudomycelium on which the blastospores are arranged exactly in the same way as with the Italian strain. Only one minor difference may be mentioned, *viz.*: the greater part of the strains of *Saccharomyces fragilis* show on malt agar slants a slightly rough appearance, whereas most strains of *Candida pseudotropicalis* are smooth. There are, however, strains of *Saccharomyces fragilis* with a fairly smooth appearance, and on the other hand we met also with strains of *Candida pseudotropicalis* with a slightly rough surface on malt agar slants.

On the whole both groups are quite identical, at least on the understanding that *Saccharomyces fragilis* can produce ascospores and *Candida pseudotropicalis* lacks this ability. We may therefore consider *Candida pseudotropicalis* as being the imperfect stage of *Saccharomyces fragilis*.

II. The five other *Saccharomyces* strains are mutually identical. They are listed in the 1937 catalogue of the "Centraalbureau" as follows:

1. *Blastodendrion macedoniense* (A. Cast.) Lang. et Guerra [4]), strain 684.
2. " " " " " " , strain 684bis.
3. *Candida macedoniensis* (A. Cast.) Berkh. var. *macedoniensoides* (A. Cast.) Westerdijk [5]).
4. *Monilia pseudotropicalis* A. Cast., strain A. T. C. C.
5. *Mycocandida pseudotropicalis* (A. Cast.) Cif. et Red.

The "Centraalbureau" received the two strains of *Blastodendrion macedoniense* from LANGERON in 1936. Originally these strains came from CASTELLANI.

Candida macedoniensis var. *macedoniensoides* was sent twice to the "Centraalbureau" by CASTELLANI, one strain in 1926 and the other in 1935.

The cultures of *Monilia pseudotropicalis*, strain A.T.C.C., and of *Mycocandida pseudotropicalis* were received respectively in 1935 from the American Type Culture Collection and in 1934 from CIFERRI and REDAELLI.

The characteristics of these five strains correspond very well with the description of *Monilia macedoniensis* and of *Monilia macedoniensis* var. *macedoniensoides*, as given by CASTELLANI [6]).

CASTELLANI's description of *Monilia macedoniensis* reads as follows: "Found in sputum. Colonies on glucose agar white, smooth. Ferments, with production of gas glucose, laevulose, galactose, saccharose and inulin, not maltose or lactose. It does not liquefy

[1]) A. CASTELLANI, Journ. of Trop. Med. and Hyg., **40**, 293, (1937).

[2]) CHR. M. BERKHOUT, „De schimmelgeslachten Monilia, Oidium, Oospora en Torula", Diss. Utrecht, (1923).

[3]) C. W. DODGE, "Medical Mycology",London, Henry Kimpton, (1936), (p. 259).

[4]) M. LANGERON et P. GUERRA, Octava Reun. Soc. argentina patol. region. Norte, Santiago del Estero, oct. 1933, Buenos Aires, 98, (1934).

[5]) List of Cultures 1932, "Centraalbureau voor Schimmelcultures" Baarn (Holland).

[6]) l. c.

gelatine. It clots milk. Some observers have described asci and consider the organism to be a *Saccharomyces*".

The description of *Monilia macedoniensis* var. *macedoniensoides* runs as follows:

"This is a common variety and has been isolated by me from the air and also from sputum. I do not consider the organism to be pathogenic. On glucose agar colonies white, smooth, composed of yeast-like cells, roundish or slightly oval, mostly 2.5—4.7 μ in diameter. In hanging-drop cultures mycelium usually absent, but occasionally a small amount of it, ramified, 2—2.5 μ in diameter, may be seen. Gelatine not liquefied, milk not clotted. Some authorities have described asci and consider the fungus to be a *Saccharomyces.*

Biochemical characters. — Among the standard carbohydrates used by me in the classification of *Monilias*, it produces gas in glucose, laevulose, galactose, saccharose, inulin, not in maltose and lactose".

From the above quoted descriptions it appears that this variety only differs from the species by its lack of ability to clot milk. As we think it inadmissible to establish a variety on this single, rather unstable character, we consider this variety as being identical with the species „*macedoniensis*".

As follows from the description of CASTELLANI, we are not first in discovering ascospore-formation in this organism.

STOVALL and BUBOLZ [1]) in 1932 also mentioned ascospores and brought this species into the genus *Endomyces*; LANGERON and GUERRA [2]) in 1938 described ascospores and classified the organism in the genus *Saccharomyces.* Undoubtedly still other scientists will have osberved ascospores in this organism.

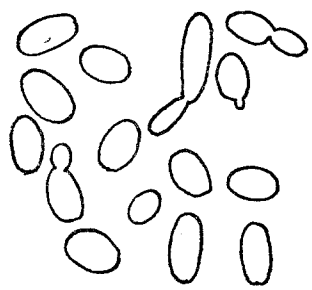

Fig. 2.

As far is known to us, nobody ever tried to compare this species with other sporogenous yeasts in order to identify it eventually with one of the known species. We think such comparative studies of great importance; the results of the investigations made are given here. For all methods employed the reader is referred to the monograph of STELLING-DEKKER [3]) on the sporogenous yeasts.

Growth on malt extract after 24 hours at 25° C:

The cells are for the greater part oval, (3.5—5) × (5—8) μ; some cells are elongated 4.5 × 12 μ; cells are single or arranged in pairs (Fig. 2).

After 6 days at 25° C the number of elongated cells has increased, (3—6) × (5.5—14)μ. A sediment and a ring are formed.

Growth on malt agar after 6 days at 25° C:

The cells are oval or elongated, (2—5) × (5—19) μ, single or in pairs.

After 40 days at 25° C the growth on malt agar slant is yellowish-white, soft, smooth and glistening.

Ascospores are easily formed on carrot slices after an incubation of some days at 25° C. The spores are oval or reniform. There are four or less spores in the ascus (Fig. 3).

Fig. 3.

Sugar-fermentation: Glucose +, fructose +, mannose +, galactose +, saccharose +, maltose —, lactose —, raffinose $^1/_3$, inulin + [4]).

Assimilation of nitrate: Negative.

Growth in a medium with ethyl alcohol as sole source of carbon: No growth.

The organism does not produce any true septated mycelium which can break up into oidia; it only reproduces by budding. Hence it

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[1]) W. D. STOVALL and A. A. BUBOLZ, Journ. of Infect. Dis., **50**, 73, (1932).
[2]) M. LANGERON et P. GUERRA, Ann. d. Parasitol. hum. et comp., **16**, 36, (1938).
[3]) l. c.
[4]) Though we never include this carbohydrate in our fermentation tests, we tried it in this case, because CASTELLANI did so.

does neither belong to the genus *Endomyces*, nor to the genus *Endomycopsis*, but is a *Saccharomyces*.

If we consider the species of *Saccharomyces* mentioned in the key to this genus drawn up by STELLING-DEKKER [1]), this organism is closest to *Saccharomyces exiguus* Reess and *Saccharomyces Mangini* Guilliermond which have the same type of sugar-fermentation (inulin was not tested). It can however easily be distinguished from these two species by the shape and the dimensions of the cells, and by the shape of the ascospores.

As was mentioned before, CASTELLANI already pointed out its belonging to the genus *Saccharomyces*, however without comparing it with other species of this genus.

As this organism could not be identified by us with one of the known species of *Saccharomyces*, it has to be considered as a new species for which we propose the name *Saccharomyces macedoniensis*.

Diagnosis of *Saccharomyces macedoniensis* n. sp.:

Cells oval or elongated, (3—6) × (5.5—14) μ, single or in pairs. A sediment and a ring are formed in malt extract. The ascospores, in the number of four or less in an ascus, are oval or reniform. Glucose, fructose, mannose, galactose, saccharose and inulin are fermented, raffinose for a third part. Nitrate is not assimilated. In a medium with ethyl alcohol as sole source of carbon no growth occurs. Growth on malt agar slant is yellowish-white, soft, smooth and glistening.

In the collection of the "Centraalbureau" there are, moreover, two cultures which have to be considered to represent the imperfect stage of *Saccharomyces macedoniensis*, *viz.*: *Candida macedoniensis* (A. Cast.) Berkh. and *Saccharomyces fragrans* Beijerinck.

The "Centraalbureau" received the former culture under the name of *Monilia macedoniensis* A. Cast. in 1920 from JOEKES. It has been studied by BERKHOUT who classified it in her newly created genus *Candida*.

The culture of *Saccharomyces fragrans* has been isolated in the "Laboratorium voor Microbiologie" at Delft by MAYER as a contamination of commercial yeast of the factory "Vrijland van der Hooge" at Schiedam (Holland). A suspension of yeast cells in water was heated at 50° C for 15 minutes and afterwards inoculated on a malt agar plate, from which a pure culture was obtained in the usual manner. BEIJERINCK mentioned *Saccharomyces fragrans* more or less extensively in various publications. The main descriptions [2]), as given by this author, are cited here in full:

"Sie [3]) besteht aus Zellen von ca. 5 à 6 μ; ist also viel kleiner wie die gewöhnliche Presshefe, welche 8 μ misst; sie vergärt Glucose, Laevulose und Rohrzucker sehr energisch zu Alkohol und Kohlensäure und erzeugt aus den beiden ersten Zuckern dazu etwas Essigäther.Sie vergärt und assimiliert Maltose, Dextrin und Stärke gar nicht. Bei der Gelatinekultur spaltet sie sich, wie viele andere Hefearten, in drei morphologische, in der Form der Kolonieen sehr verschiedene Varietäten, wovon eine aus gewöhnlichen ellipsoidischen, eine andere aus langfadenförmigen Zellen besteht, welche sich aber, eben wie die dritte Zwischenform, physiologisch identisch verhalten."

[4]) "Je l'appelle *Saccharomyces fragrans* puisque lors de sa croissance en présence de glucose elle dégage un peu d'acétate d'éthyle. Elle mérite quelque attention à cause de sa grande vitalité et de son très haut maximum de croissance, trouvé un peu au-dessus de 41° C."

The strain of *Saccharomyces fragrans* isolated by MAYER corresponds very well to BEIJERINCK's not very extensive description. BEIJERINCK did not describe ascospore-formation and, although this strain was tested on this ability immediately after its isolation, neither MAYER, nor we observed asci. In all other respects it is identical with *Saccharomyces macedoniensis*. Besides it has two more characteristics, not inserted in our description of *Saccharomyces macedoniensis* [5]) *viz.*: the development of a slight odour

[1]) l. c.

[2]) M. W. BEIJERINCK, Verzamelde Geschriften, III, 132 and 133, (1921) from Centralbl. f. Bakt. und Parasit. II, **1**, 221, 265, 329, (1895).

[3]) *Saccharomyces fragrans*.

[4]) M. W. BEIJERINCK, Verzamelde Geschriften, IV, 64, (1921) from Archives Néerlandaises des Sciences Exactes et Naturelles, Sér. II, **7**, 212, (1901).

[5]) As the description of *Saccharomyces macedoniensis* was formulated in analogy to those in STELLING-DEKKER's monography.

of fruit ether and a good growth at relatively high temperatures (41° C). MAYER used this latter characteristic in isolating *Saccharomyces fragrans*. We could confirm the presence of these two characteristics in all our strains of *Saccharomyces macedoniensis* and in the strain of *Candida macedoniensis*.

As BEIJERINCK did not describe ascospore-formation with his *Saccharomyces fragrans*, he incorrectly placed this organism in the genus *Saccharomyces*; for this reason we cannot accept the species name for the non-spore-forming stage (*Candida macedoniensis*); for the same reason we neither can accept it for the spore-forming stage (*Saccharomyces macedoniensis*). We only have to register the name *Saccharomyces fragrans* as a synonym of *Candida macedoniensis*.

In comparing the strains of *Candida macedoniensis* with those of *Saccharomyces macedoniensis*, we also made slide cultures in order to study the development of pseudomycelium with blastospores. Not only did both sets of cultures appear to be quite identical — as had been expected — but they showed, moreover, a striking resemblance with the slide cultures of *Saccharomyces fragilis* and *Candida pseudotropicalis* (cf. Fig. 1). Besides we compared both groups of organisms ("*fragilis-pseudotropicalis*" and "*macedoniensis*") in other respects and found them to be closely related. Both have the same appearance on slide cultures; both are able to ferment glucose, fructose, mannose, galactose, saccharose, inulin and raffinose for $^1/_3$, but not maltose; both can develop a slight odour of fruit ether and grow at a relatively high temperature (41° C).

The difference between these two species rests upon the ability of the "*fragilis-pseudotropicalis*" group to ferment lactose, whereas "*macedoniensis*" lacks this capacity. Besides — as was mentioned above — *Saccharomyces fragilis* generally has a somewhat rough appearance on malt agar, while the cultures of *Candida pseudotropicalis* show a more smooth growth and all strains of *Saccharomyces macedoniensis* and *Candida macedoniensis* are smooth on malt agar. Though the appearance of cultures is only a characteristic of secondary order, we still think it worth mentioning.

The ability of a yeast to ferment lactose is a very noteworthy and distinct character, and is generally accepted in yeast-taxonomy to offer sufficient ground for the differentiation of species. Though we consider both organisms as being very closely related, we want them for this reason to be kept separated into two species.

In view of the above mentioned it is no wonder to find that the great similarity of both species has given rise to errors in their identification. For instance, two cultures, labeled *Monilia pseudotropicalis* and *Mycocandida pseudotropicalis*, respectively appeared to be identical with *Saccharomyces macedoniensis*, whereas on the other hand a culture, sent in as *Mycotorula macedoniensis*, proved to be *Candida pseudotropicalis*.

The origin of the culture of *Monilia pseudotropicalis* we received from the American Type Culture Collection is not known to us. *Mycocandida pseudotropicalis* has been described by CIFERRI and REDAELLI [1]) who received the culture from CASTELLANI. They apparently did not test the sugar-fermentation, but published the data, given by CASTELLANI. In the same paper the two Italian investigators described *Mycotorula macedoniensis* after an authentic culture of CASTELLANI; they referred likewise in this case to this author for the results of the sugar fermentation. After our investigations, however, the *Mycotorula macedoniensis* we received from CIFERRI and REDAELLI, is identical with *Candida pseudotropicalis*. It seems probable that a mistake will have been made in labeling these cultures, owing to their great similarity.

B. We found two ascosporogenous strains belonging to the genus *Hansenula*:

1. *Monilia javanica* Went et Prinsen Geerligs. This yeast has been isolated by WENT and PRINSEN GEERLIGS from "raggi", a kind of rice cake, made by indigenous people in the Netherlands East-Indies. The "Centraalbureau" received this strain in 1912 from WENT. WENT and PRINSEN GEERLIGS [2]) give the following description:

"Makroskopisches Äussere. Wenn man in einen Kolben mit zuckerhaltiger Nährflüssigkeit einige Zellen dieser Hefe bringt, so bleiben diese auf der Oberfläche der

[1]) l. c.

[2]) F. A. F. C. WENT en H. C. PRINSEN GEERLIGS, Meded. v. h. Proefstation v. Suikerriet "West Java" te Kagok-Tegal, N. 13, (1894); Archief v. d. Java-suikerindustrie (1894); Verh. Kon. Akad. v. Wetensch. te Amsterdam, **4**, Sect. 2, N. 2, (1895).

Flüssigkeit schwimmen, und bilden, wenn sie sich vermehren, nach 1 bis 2 Tagen eine Kahmhaut. Darauf wird die Kahmhaut gekräuselt und steigt auch an die Wand des Kolben etwas über die Flüssigkeit empor. Zugleicherzeit sinken eine Anzahl Hefezellen bis auf den Boden des Gefässes. Bald tritt jetzt Gährung ein, grosse Kohlensäureblasen heben die Kahmhaut empor und zerbrechen sie zuletzt; nach etwa 10 Tagen ist die Gährung abgelaufen.

Zieht man die Hefe auf Agar-agarplatten, denen Zucker und die nöhtigen Nährsalze zugesetzt sind, so breitet sie sich in dünner Schicht rasch auf der Oberfläche des Agar-agars aus, wobei der Rand, wo die Kolonie weiter wächst, erst eine schwach gebogene Linie ist; später entstehen aber Ausstülpungen an diesem Rande, sodass die ganze Kolonie wie mit Franzen umgeben ist. Bei schwacher Vergrösserung scheinen diese Franze aus durcheinander geschlungenen Pilzfäden zu bestehen .....

Mikroskopisches Aussehen.

Betrachtet man die Hefe aus der Kahmhaut, aus der Mitte der Kolonien auf Agar oder vom Boden der Kulturkolben, so ergeben sich Bilder wie in Fig. 1*a*—*i* dargestellt, also runde oder birnförmige Hefezellen, welche sich in der gewöhnlichen Art durch Sprossung vermehren, nur bisweilen etwas unregelmässige Formen (*d*, *i*) annehmen und sogar dann und wann zu Fäden auswachsen können (*e*, *f*). Die Zellen bleiben ziemlich lange an einander befestigt.

Betrachten wir die Franzen der Agarkolonien, oder besser noch die Auswüchse der Reiskörner, so finden wir ein verschlungenes Gewebe von Pilzfäden, sodass Körper entstehen, wie sie in Fig. 2 im optischen Querschnitt dargestellt sind .... An der Peripherie sprossen aus diesen Pilzfäden Hefezellen hervor ....

Systematische Stellung. Aus dem Vorgehenden zeigt sich, dass dieser Hefepilz wenig Übereinstimmung hat mit den Arten der Gattung *Saccharomyces*, wenn man diese im Sinne HANSEN's auffasst. Überzeugend stellte sich das heraus, als die Hefe auf feuchten Gipsblöckchen gezogen wurde und sich trotzdem *keine endogenen Sporen* bildeten. Es scheint uns, dass diese Hefe nur ein Entwickelungszustand irgend eines höheren Pilzes ist; diesen Pilz zu finden, ist uns aber nicht gelungen. Da die Hefe nun doch einen Namen haben muss, so wird sie wohl am Besten untergebracht in die Gattung *Monilia*, wo so viele Formen hingestellt werden, mit denen man eigentlich nicht viel zu machen weiss. Diese Spezies nennen wir *Monilia javanica.*

Physiologische Eigenschaften .... Als Stickstoffnahrung wird am liebsten Pepton benützt, dann kommen Asparagin und Ureum, weiter Nitrate und am wenigsten gern werden Ammoniumsalze (sowohl Sulfate wie Chloride) aufgenommen. Nitrite können gar nicht als Stickstoffnahrung dienen ....

Gährung findet statt in Lösungen von Dextrose, Laevulose, Maltose, Raffinose und Saccharose (welche erst von der Hefe invertirt wird), dagegen nicht von Lactose ....".

BERKHOUT [1]) studied the culture the "Centraalbureau" received from WENT. She observed ascospore-formation and rightly placed this yeast because of the hat-shaped form of the spores and other characters in the genus *Willia* (*Hansenula*). But after her opinion the culture does not correspond to the description of *Monilia javanica* by WENT and PRINSEN GEERLIGS for the following three reasons: 1. She observed ascospores, whereas WENT and PRINSEN GEERLIGS tested this character on blocks of plaster with negative results; 2. No bundles of threadlike cells were observed by her; 3. She never saw any conidia giving rise to threadlike cells. BERKHOUT namely interpreted in this way the statement of the phenomenon by WENT and PRINSEN GEERLIGS that sometimes the round or pear-shaped cells can grow out to threadlike cells.

When we critically consider these three points, we firstly have to remark that WENT and PRINSEN GEERLIGS tested the ascospore-formation of the culture only on blocks of plaster. This fact does not exclude the possibility that they could have had positive results on other media. We, for instance, observed ascospore-formation in rather old malt agar cultures.

2. The bundles of threadlike cells ("verschlungenes Gewebe von Pilzfäden") mentioned in the publication of WENT and PRINSEN GEERLIGS are apparently coremia. This also is quite clear if we examine Fig. 2 of the publication of these authors. Pseudomyce-

[1]) l.c.

lium developing yeasts very often form coremia. On slide cultures of the strain in question they frequently were observed.

3. WENT and PRINSEN GEERLIGS observed sometimes the round cells not producing round cells again by budding, but threadlike ones. BERKHOUT took these threadlike cells for germ tubes which arise from conidia (the round cells). We however cannot subscribe to this interpretation. From the description of WENT and PRINSEN GEERLIGS it is clear that they observed the formation of a pseudomycelium. We came also upon an abundant development of pseudomycelium in our slide cultures.

From the above we may conclude that the culture in the collection of the "Centraalbureau", received from WENT in 1912, corresponds very well to the description of *Monilia javanica*. On the other hand we agree with BERKHOUT who found the organism to belong to the genus *Hansenula*. We could identify it with *Hansenula anomala* (Hansen) Sydow [1]). This is a rather common yeast, isolated for the first time by HANSEN from brewery yeast in 1891.

2. The second strain belonging to the genus *Hansenula* was sent to the "Centraalbureau" by NIÑO in 1933 as *Monilia* species. It has been isolated by MOLLE from "intertrigo blastomicetico". This strain easily forms hat-shaped ascopores on malt-agar. It proved to be identical with *Hansenula javanica* (Groenewege) Dekker [2]). GROENEWEGE has isolated this organism in 1920 from moulded sheet-rubber. This species has since been frequently isolated from different sources.

Studying our anascosporogenous strains we also met with the imperfect stage of *Hansenula javanica*, *viz. Candida pelliculosa* Red. This organism has been isolated in 1925 by REDAELLI [3]) from a case of pulmonary tuberculosis. *Candida pelliculosa* corresponds in every respect, except ascospore-formation, to *Hansenula javanica*, but it too shows a great likeness with *Hansenula anomala*. The main difference between these two *Hansenula* species lies in the fact that *Hansenula javanica* has only round or oval cells in young malt extract- or malt agar cultures, whereas *Hansenula anomala* shows next to these round or oval cells in addition long cells, or putting it somewhat differently: *Hansenula javanica* does not produce pseudomycelium in young malt extract- or malt agar cultures, whereas *Hansenula anomala* already develops pseudomycelium in young cultures. The other characters of both species are the same.

Cultures of both *Hansenula* species and of *Candida pelliculosa* were also identical in their development on slides.

In conclusion we may say that *Hansenula javanica* represents the perfect stage of *Candida pelliculosa*, both showing the same cell shape and size in young malt extract- and malt agar cultures.

C. One of the strains we found to be sporogenous appeared to belong to the genus *Debaryomyces*. We received this strain, labeled *Myceloblastanon gifuense* Taniguchi, in 1935 from OTA.

TANIGUCHI [4]) isolated from a "*Erosio interdigitalis blastomycetica*" of labourers of a paper factory in the province Gifu in Japan a yeast which he described as *Myceloblastanon gifuense*. After this description the organism is an asporogenous yeast, able to ferment glucose, fructose, mannose and maltose, not galactose, saccharose or raffinose. The cells are oval or oblong; often filaments, consisting of very long cells, are formed.

This description of *Myceloblastanon gifuense* does not correspond at all with the characters of the culture we received under the same name from OTA. OTA's organism does not show any long cells, but only round or oval cells, neither does it have any fermentative power.

Hence it follows that the organism sent by OTA is not *Myceloblastanon gifuense* and therefore incorrectly bears this name. OTA's organism develops ascospores, especially

[1]) cf. N. M. STELLING-DEKKER, l. c., (p. 406).

[2]) cf. N. M. STELLING-DEKKER, l. c., (p. 427).

[3]) P. REDAELLI, "I miceti come associazione microbica nella tubercolosi polmonare cavitaria", Pavia (1925).

[4]) Y. TANIGUCHI, Jap. Journ. of Med. Sciences, Trans XIII, Dermatol. and Urol., **1**, 75, (1927).

on carrot-slices. The ascus contains only one spore which has a verrucous wall. Copulation has been observed, preceding the ascus-formation. It undoubtedly belongs to the genus *Debaryomyces* and is identical with *Debaryomyces Matruchoti* Grigoraki et Péju [1]). The latter species has been isolated by the authors from the stool of a patient suffering from "helminthiasis". In the course of time the "Centraalbureau" received several strains of this species for identification, most of them from human sources.

Summary

In a systematic study of about 300 supposedly anascosporogenous yeast cultures it was found that nine strains actually did form ascospores. A further investigation of these strains, together with a comparative study of evidently closely related asporogenous strains, led to the following results.

Mycocandida pinoyisimilis (A. Cast.) Red. et Cif. var. *Citelliana* Red. et Cif. proved to be identical with *Saccharomyces fragilis* Jörgensen.

Monilia pseudotropicalis A. Cast. = *Candida pseudotropicalis* (A. Cast.) Basgal has to be considered as the imperfect stage of *Saccharomyces fragilis* Jörgensen.

Monilia macedoniensis A. Cast. = *Blastodendrion macedoniense* (A. Cast.) Lang. et Guerra, as well as the variety *macedoniensoides* [= *Candida macedoniensis* (A. Cast.) Berkh. var. *macedoniensoides* (A. Cast.) Westerdijk], should in future be designated as: *Saccharomyces macedoniensis* Diddens et Lodder.

The yeast incorrectly named by Beijerinck: *Saccharomyces fragrans* proved to be the imperfect stage of *Saccharomyces macedoniensis* Diddens et Lodder. Hence the correct designation of the species in question appears to be: *Candida macedoniensis* (A. Cast.) Berkh.

Monilia javanica Went et Prinsen Geerligs proved to be identical with *Hansenula anomala* (Hansen) Sydow.

A *Monilia* species isolated by Molle from „intertrigo blastomicetico" proved to be identical with *Hansenula javanica* (Groenewege) Dekker.

Candida pelliculosa Red. is the imperfect stage of the last mentioned species.

A strain received from Ota, and labeled: *Myceloblastanon gifuense* Taniguchi — which, however, did not answer the diagnosis of this species — proved to be identical with *Debaryomyces Matruchoti* Grigoraki et Péju.

[1]) cf.: N. M. Stelling-Dekker, l. c., (p. 467).