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A NEW SYSTEM OF CLASSIFICATION OF THE GENUS *DEBARYOMYCES* BASED ON ACID PRODUCTION

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Yeasts belonging to the genus *Debaryomyces* play an important role in the preservation of food. They are different from other yeasts ecologically because of their wide distribution and high salt tolerance, and especially because of their common occurrence in brined, pickled, and other meat products.[1] On the other hand, they differ physiologically from the other true yeasts in the production of flavin pigment.[2] Although they are of much interest in this respect, their usefulness has not been recognized in the fermentation industry.

Among the taxonomic studies of *Debaryomyces*, which have hitherto been made, systems have been devised by Césari & Guilliermond (1920),[3] Konokotina & Krassilnikov (1929),[4] Stelling-Dekker (1931),[5] Lodder (1934),[6] and in the field of medicine, by Dodge (1935)[7]; and recently another new system was presented by Naganishi (1948).[8]

The ability of sugar-fermentation has been generally employed as an important key in classifying the true yeasts. But, the fermentation ability of all species belonging to *Debaryomyces*, with a few exceptions, is unstable and variable, as already pointed out by Naganishi (1940).[9] Therefore, the classification of *Debaryomyces* presented by Konokotina & Krassilinikov, Stelling-Dekker, Lodder, and Dodge, based on sugar-fermentation, cannot be considered applicable. Naganishi, in his system, while dividing *Debaryomyces* into "yellowish strain group"* and "non-yellowish strain group",

* The species belonging to this group produce a lemon-yellow pigment on the modified Hayduck medium and change the color of the medium to yellow, glimmering with green fluorescence, whereas the other group produces no yellow pigment on the same medium, and is generally fermentative.

did not employ the sugar-fermentation test for the identification of species in the former group.

As regards the specific characteristics of *Debaryomyces*, Zopf (1889)[10] reported the production of oxalic acid, and the same fact was demonstrated by Perwozwansky (1930).[11] The species used in their studies, *Saccharomyces Hansenii*, was amended to *Debaryomyces tyrocola* var. *Hansenii* (Zopf) Dekker. Naganishi (1940)[12] estimated the acidity of Koji water cultures using a large number of the species of this genus.

Taking these facts into consideration, the author investigated the ability of yeasts to produce acid from various kinds of sugar, and found it useful in the classification of *Debaryomyces*. The acid production test has been applied widely in the classification of bacteria, though never to that of true yeast. However, in the case of asporogenous yeast, the applicability of the test was ascertained by Castellani (1913),[13] who applied it to the genus *Monilia*; and Martin, et al. (1940),[14] to the genus *Candida*. Strong acid production by the genus *Brettanomyces* is also given considerable taxonomic weight by Custer (1940).[15]

In this paper, a new classification system of *Debaryomyces* based on acid production will be presented, together with the description, not only of its action on several sugars which have been previously tested in order to be employed for the identification of species or group, but also of the experimental conditions about "standard test" in detecting acid production.

Strains of *Debaryomyces* Used in the Experiment

Of the 31 strains used in this study, 18 were isolated and identified by the author, while undetermined strains are merely described as *Debaryomyces* sp. The yeasts that were tested, are listed in the following table.

Table 1. Strains of *Debaryomyces* used.

1. *Debaryomyces membranaefaciens* Naganishi.
2. *Debaryomyces membranaefaciens* var. *Zingiberi* Otani.
3. *Debaryomyces membranaefaciens* var. hollandicus Lodder.
4. *Debaryomyces* sp. C_2 (*D. membranaefaciens* type).
5. *Debaryomyces* sp. O_9 (*D. membranaefaciens*).
6. *Debaryomyces* sp. P_2 (*D. tyrocola* type).

7. *Debaryomyces japonicus* Naganishi.
8. *Debaryomyces* sp. C_4 (*D. tyrocola* type).
9. *Debaryomyces* sp. S_3 („ „).
10. *Debaryomyces* sp. C_1 („ „).
11. *Debaryomyces* sp. Q_3 („ „).
12. *Debaryomyces* sp. Q_4 („ „).
13. *Debaryomyces miso* Mogi.
14. *Debaryomyces miso* var. α Mogi.
15. *Debaryomyces* sp. Y_1.
16. *Debaryomyces Klöckeri* Guilliermond et Péju.
17. *Debaryomyces* sp. S_4 (*D. Klöckeri* type).
18. *Debaryomyces* sp. A_2 (*D. Matruchoti* type).
19. *Debaryomyces manchuricus* Naganishi.
20. *Debaryomyces globosus* Klöcker.
21. *Debaryomyces* sp. V_1.
22. *Debaryomyces* sp. X_3.
23. *Debaryomyces sake* Saito et Oda.
24. *Debaryomyces Matruchoti* Grigoraki et Péju.
25. *Debaryomyces* sp. S_2 (D. n. sp. unpublished)
26. *Mycoderma* sp. Q. Naganishi evaluated this yeast as *Debaryomyces*.
27. *Debaryomyces* sp. W_1.
28. *Debaryomyces tyrocola* var. *Hansenii* (Zopf) Dekker.
29. *Debaryomyces orientalis* Naganishi.
30. *Debaryomyces* sp. R_4.
31. *Debaryomyces Guilliermondii* var. *nova-zeelandicus* Lodder.

Action of *Debaryomyces* on Sugars

a) *Fermentation test*:—As shown in the classification of Stelling-Dekker and of Lodder, there are many species which have no fermentation ability. According to Naganishi (1940), they are found to have the ability, in the case of fresh yeast cells, and a number of notices have been given by him about the procedure of the fermentation test. The author has frequently experienced that, even with fresh cells, there occurs no fermentation, or, if it does, it is very erratic. Therefore, the fermentation reaction of *Debaryomyces* (excepting the fermentative group by Naganishi) is considered as insufficient for the basis of classification.

b) *Sugar-assimilation test*:—Lodder[16] has applied the assimilability of sugar as a key to classifying non-fermentative species, which belong to *Torulopsidaceae*, and furthermore, Diddens & Lodder (1942)[17] have extended its employment to all species of *Mycotoruloideae*. The author made an investigation to determine whether this test could be adopted as a key to classifying

Debaryomyces. Naganishi (1934)[18] studied the ability of sugar-assimilation with this genus, using the modified Hayduck's medium. The author repeated a similar test on some selected species using Lodder's medium. The basal medium consists of 0.5 % $(NH_4)_2SO_4$, 0.1 % KH_2PO_4, 0.01 % $MgSO_4 \cdot 7H_2O$. To this solution 2 % each of the test sugars were added. Each 5 cc. of the medium was inoculated with two drops of yeast suspension in sterilized water, and the cultures were examined after five and ten days at 25°C. The results are shown in Table 2.

Table 2. Sugar-assimilation test of several species.

Strains \ Sugar	glucose	fructose	mannose	galactose	sucrose	maltose	lactose	raffinose	xylose	arabinose	rhamnose
D. sake	+	+	++	++	++	++	−	+	+	++	−
D. miso	++	++	++	++	++	++	−	+	+	++	−
D. Klöckeri	+	+	+	++	+	++	−	+	+	++	−
D. orientalis	+	+	+	+	+	+	+	+	+	++	−
D. japonicus	++	++	+	+	++	+	−	++	+	++	−
D. sp. R_4	+	+	+	+	+	+	+	+	+	+	−
D. sp. C_2	++	++	++	++	++	++	−	+	+	++	−
D. sp. Q_3	+	+	+	+	+	+	−	+	+	++	−
D. sp. S_3	++	++	++	++	++	++	−	+	+	++	−
D. sp. C_1	++	+	++	++	++	++	−	+	+	+	−

No remarkable differences were found in their ability to assimilate the sugar, with the exception of lactose, although the test yeasts showed an obvious difference from ordinary yeasts in the case of pentose-assimilation. Thus, the sugar assimilability is considered to be of no significance in the taxonomy of this genus, with the exception that lactose-assimilation seems to be helpful in some cases.

c) *Acid production test*:—The test methed was established as follows:

On Acid Production of *Debaryomyces*

a) *Relation between pH and acid production.*

In establishing the standard test by detecting acid-production,

it is necessary to know the relation between acidity and pH during the culture of *Debaryomyces*. Accordingly, the author experimented with typical species of this genus for acid-producing ability, fate, and pH change.

The test yeast: The fermentable species—*D. manchuricus* Naganishi and *D. globosus* Klöcker—and the unfermentable species—*D.* sp. P_2, *D.* sp. S_4, and *D. tyrocola* var. *Hansenii*—were used.

The experimental procedure: The basal medium was prepared as follows: 5% glucose, 0.25% peptone, and 1.0% NaCl were dissolved in distilled water, and the pH was adjusted at 7.0. 90 cc. of basal medium in a 100 cc. Erlenmeyer flask was inoculated with the test yeast, cultured on Sabouraud agar at 25°C for a few days, and allowed to stand at 25°C. The acidity of 5 or 10 cc. of the sample was estimated by titrating with 0.01N NaOH, using phenolphthalein as an indicator. The acidity is shown in the following table by the number of cc. of 0.1N NaOH necessary to neutralize the acid in 100 cc. of medium. The pH value was measured electrometrically.

The results obtained: Both of the fermentable species were strong acid producers. Acidity-curves distinguished them from the unfermentable species (Fig. 1), and a similar tendency was observed in the pH curves (Fig. 2). Among the species of each group, there

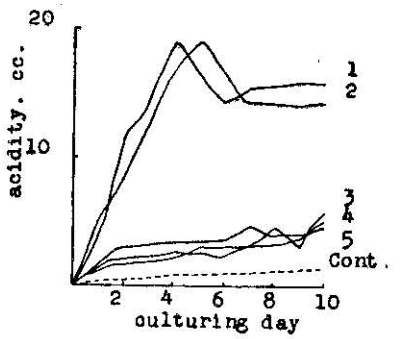

Figure 1. Change of acidity in the culture of some species of *Debaryomyces*.

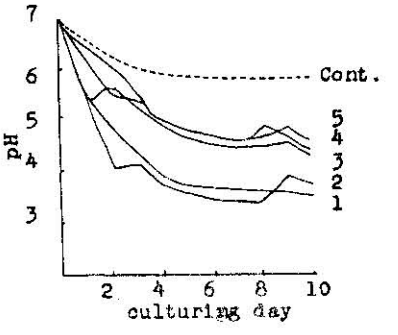

Figure 2. Change of pH by acid production of some species of *Debaryomyces*.

1. *Debaryomyces globosus*.
2. *Debaryomyces manchuricus*.
3. *Debaryomyces* sp. S_4.
4. *Debaryomyces* sp. P_2.
5. *Debaryomyces tyrocola* var. *Hansenii*.

is little difference in acidity- and pH-curves. Since the pH value of the culture medium exhibits a tendency to decrease below a pH of 5.0 within 2-4 days, its degree of change will be available for the detection of acid production.

b) *Relation between the acid-producing ability and its pH in various concentrations of sugar.*

This experiment was carried out to decide a sugar concentration strong enough to have a distinct effect on the indicator, though most of *Debaryomyces* do not need as great a quantity of sugar as the alcohol yeasts as a source of energy.

The test yeast: Since the previous experiment showed no marked difference among the strains in each group, the following two typical species, *D. globosus* Klöcker and *D.* sp. C_2, were used.

The experimental procedure: The concentration of glucose solutions used, were 0.5, 1.0, 2.0, 3.0, and 5.0%, respectively. All other conditions were the same as in the previous experiment.

The results obtained: *D. globosus* showed the maximum acidity within 2-4 days. The acidity then decreased gradually (Fig. 3), and the pH dropped below 5.0 (Fig. 4). In the case of *D.* sp. C_2, the acidity gradually increased, and the pH decreased independently of sugar concentration (Fig. 5). Hence, 0.5-2.0% sugar solution is considered to be adequate.

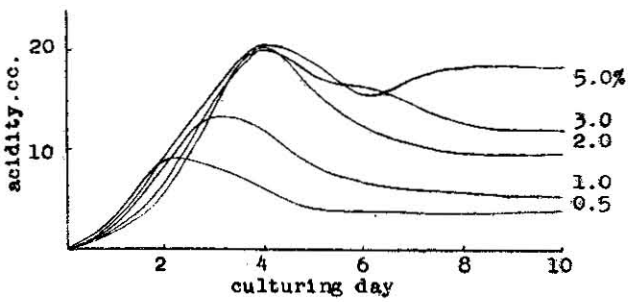

Figure 3. Change of acidity in the cultures of *D. globosus* with various concentrations of glucose.

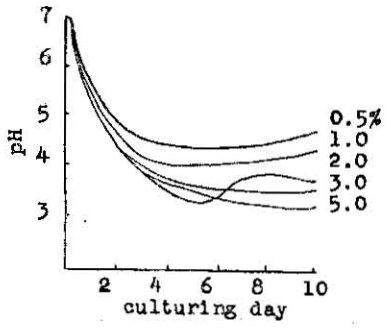

Figure 4. pH change in the cultures of *D. globosus* with various concentrations of glucose.

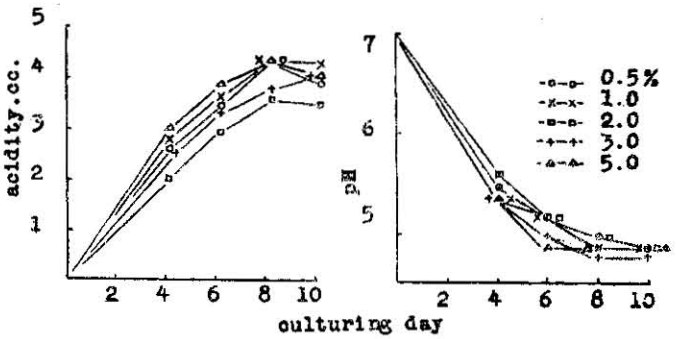

Figure 5. Changes of pH and acidity in the cultures of *D.* sp. C_2 (*D. membranaefaciens* type) with various concentrations of glucose.

c) *Establishment of standard test in acid production.*

Basal medium for standard test: Barsiekow's medium is usually used for the acid production test on bacteria, but it is unsuitable for the identification of yeast. The test medium for this purpose requires a suitable ratio between nitrogen and carbone; and addition of nutrient salts, such as phosphates, exhibits a tendency to delay acid production. Therefore, the basal medium was prepared as follows: 0.25 % peptone, 1.0 % NaCl, and 2 % test sugar were dissolved in distilled water, and 0.2 % Bromthymol blue solution was added to facilitate the judgment of acid production (12 cc. of the indicator to 1 liter of basal medium). The pH was then adjusted at 7.0. 3 to 4 cc. of the medium were placed in a small test tube and sterilized by ordinary procedure.

Experimental procedure: In general, American-type Sabouraud agar was used as the pre-culture medium. No difference from wort agar was perceived during the course of this experiment. During the incubation period, two day- and five day-cultures gave a consistent result with a slight difference in detecting-days. The results of comparison between the author's method with that of Martin (Table 3) showed that there was a tendency for delayed acid production in the Martin's method.

Table 3. Comparison of acid production test between Martin's method and the author's.

Strains / Sugars		*D. sake*						*D.* sp. R_4							*D.* sp. Q_3						
		1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	3	4	5	6	7 days
Glucose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	M	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+
Maltose	A	−	−	−	−	−	−	−	−	±	+	+	+	+	−	−	±	±	±	±	+
	M	−	−	−	−	−	−	−	−	−	±	+	+	+	−	−	−	+	+	+	+
Sucrose	A	−	+	+	+	+	+	±	+	+	+	+	+	+	−	±	+	+	+	+	+
	M	−	−	±	±	+	+	−	−	±	+	+	+	+	−	−	±	+	+	+	+
Lactose	A	−	−	−	−	−	−	−	−	±	±	+	+	+	−	−	−	−	−	−	−
	M	−	−	−	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	−

Note: A−The author's method, M−Martin's method.

Color and pH *change in basal medium*: In order to determine a color and pH change during the cultivation, the relation between them was observed on 0.25 % peptone water over a wide range of pH. The relations are as follows:

Color	pH	Degree of acidity
lemon yellow	below 5.4	++
slight greenish-yellow	ca. 5.4-5.5	+
light green	ca. 5.6-5.8	±
light blue	above 5.9	−

d) *Acid production test with various kinds of sugar.*

On 31 strains of this genus, the acid production test was carried out by the standard method, using glucose, maltose, sucrose, lactose, raffinose and galactose, with the following results (Table 4).

Table 4. The results of acid production test.

D. tyrocola var. *Hansenii*

*	1	2	3	4	5	6 day
D	−	++	++	++	++	++
M	−	−	−	++	++	++
S	−	+·	++	++	++	++
L	−	−	−	++	++	++
R	−	−	−	++	++	++
G	−	+	++	++	++	++

D. Guilliermondii var. *nova-zeelandicus*

	1	2	3	4	5	6
D	−	+	++	++	++	++
M	−	−	−	++	++	++
S	−	+	++	++	++	++
L	−	−	−	−	++	++
R	−	−	++	++	++	++
G	−	++	++	++	++	++

D. orientalis

	1	2	3	4	5	6
D	−	++	++	++	++	++
M	−	−	−	++	++	++
S	−	++	++	++	++	++
L	−	−	−	+	++	++
R	−	−	−	−	+·	++
G	−	+	++	++	++	++

D. sp. R_4

	1	2	3	4	5	6
D	−	+	++	++	++	++
M	−	−	−	+	++	++
S	··	·+	+·	++	++	++
L	−	−	+	+	++	++
R	−	−	+·	++	++	++
G	−	+	++	++	++	++

D. membranaefaciens

	1	2	3	4	5	6
D	+·	++	++	++	++	++
M	−	++	++	++	++	++
S	−	++	++	++	++	++
L	−	−	−	−	−	−
R	−	++	++	++	++	++
G	−	++	++	++	++	++

D. membranaefaciens var. *Zingiberi*

	1	2	3	4	5	6
D	−	++	++	++	++	++
M	−	−	+	++	++	++
S	−·	−	+	++	++	++
L	−	−	−	−·	−	−
R	−	−	−	−	+·	++
G	−	++	++	++	++	++

D. membranaefaciens var. *hollandicus*

	1	2	3	4	5	6
D	−	++	++	++	++	++
M	−	−	+	++	++	++
S	−	++	++	++	++	++
L	−	−	−	−	−	−
R	−	−	++	++	++	++
G	−	++	++	++	++	++

D. sp. O_9

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	−	+	++	++	++	++
S	++	++	++	++	++	++
L	−	−	−	−	−	−
R	+	++	++	++	++	++
G	++	++	++	++	++	++

D. sp. C_2

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	+	++	++	++	++	++
S	++	++	++	++	++	++
L	−	−	−	−	−	−
R	−	+	++	++	++	++
G	++	++	++	++	++	++

D. miso

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	−	+·	+	++	++	++
S	−	−	++	++	++	++
L	−	−	−	−	−	−
R	−	−	+·	++	++	++
G	−	++	++	++	++	++

D. miso var. *α*

	1	2	3	4	5	6
D	−	++	++	++	++	++
M	··	··	+	++	++	++
S	−	++	++	++	++	++
L	−	−	−	−	−	−
R	−	−	+	++	++	++
G	−	++	++	++	++	++

D. japonica

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	+	++	++	++	++	++
S	+	++	++	++	++	++
L	−·	−	−	−	−	−
R	−	+	++	++	++	++
G	+	++	++	++	++	++

D. sp. C_1

	1	2	3	4	5	6
D	+	++	++	++	++	++
M	−	−	−	−	++	++
S	+	++	++	++	++	++
L	−	−	−	−	−	−
R	−·	··	−	+	++	++
G	+	++	++	++	++	++

D. sp. C_4

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	−	−	+·	+	++	++
S	+	++	++	++	++	++
L	−	−	−	−	−	−
R	−	−	+·	+	++	++
G	+	++	++	++	++	++

D. sp. S_3

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	−·	+	+	+	++	++
S	−·	+·	+·	++	++	++
L	−	−	−	−	−	−
R	−	−	+	+	++	++
G	++	++	++	++	++	++

* Note: The test sugar.

D.−glucose S.−sucrose R.−raffinose
M.−maltose L.−lactose G.−galactose

D. sp. P_2

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	+	+	+	+	+	+
S	+	+	+	+	+	+
L	−	−	−	−	−	−
R	+	+	+	+	+	+
G	+	+	+	+	+	+

D. sp Q_4

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	+	+	+	+	+
S	−	−	+	+	+	+
L	−	−	−	−	−	−
R	−	−	−	−	+	+
G	+	+	+	+	+	+

D. Klöckeri

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	−	−	+	+
S	+	+	+	+	+	+
L	−	−	−	−	−	−
R	−	−	−	−	+	+
G	+	+	+	+	+	+

D. sp. V_1

	1	2	3	4	5	6
D	−	−	+	+	+	+
M	−	−	−	−	+	+
S	−	−	+	+	+	+
L	−	−	−	−	−	−
R	−	−	−	−	−	−
G	+	+	+	+	+	+

D. sake

	1	2	3	4	5	6
D	−	+	+	+	+	+
M	−	−	−	−	−	−
S	−	−	−	+	+	+
L	−	−	−	−	−	−
R	−	−	−	−	−	−
G	−	+	+	+	+	+

D. sp. W_1

	1	2	3	4	5	6
D	−	+	+	+	+	+
M	−	−	−	−	−	−
S	−	−	−	−	−	−
L	−	−	−	−	−	−
R	−	−	−	−	−	−
G	−	−	−	+	+	+

D. sp. Y_1

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	−	+	+	+
S	−	+	+	+	+	+
L	−	−	−	−	−	−
R	−	−	−	−	−	+
G	−	+	+	+	+	+

D. manchuricus

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	+	+	+	+
S	+	+	+	+	+	+
L	−	−	−	−	−	−
R	−	−	+	+	+	+
G	−	+	+	+	+	+

D. sp. S_4

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	+	+	+	+	+
S	+	+	+	+	+	+
L	−	−	−	−	−	−
R	−	+	+	+	+	+
G	+	+	+	+	+	+

D. sp. X_3

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	+	+	+	+
S	−	+	+	+	+	+
L	−	−	−	−	−	−
R	−	−	−	−	−	+
G	+	+	+	+	+	+

Mycoderma sp. Q

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	−	−	−	−
S	−	−	+	+	+	+
L	−	−	−	−	−	−
R	−	−	−	−	−	−
G	−	−	+	+	+	+

D. sp. Q_3

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	+	+	+	+
S	−	−	+	+	+	+
L	−	−	−	−	−	−
R	−	−	−	+	+	+
G	+	+	+	+	+	+

D. globosus

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	+	+	+	+
S	+	+	+	+	+	+
L	−	−	−	−	−	−
R	+	+	+	+	+	+
G	−	−	−	−	−	−

D. sp. A_2

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	−	+	+	+
S	−	+	+	+	+	+
L	−	−	−	−	−	−
R	−	−	+	+	+	+
G	+	+	+	+	+	+

D. Matruchoti

	1	2	3	4	5	6
D	−	+	+	+	+	+
M	−	−	−	−	−	−
S	−	−	−	−	−	+
L	−	−	−	−	−	−
R	−	−	−	−	−	+
G	−	−	+	+	+	+

D. sp. S_2

	1	2	3	4	5	6
D	+	+	+	+	+	+
M	−	−	−	−	−	−
S	−	+	+	+	+	+
L	−	−	−	−	−	−
R	−	+	+	+	+	+
G	−	−	−	−	−	−

A NEW CLASSIFICATION SYSTEM FOR *DEBARYOMYCES*

The 31 strains tested can be divided into the following four groups, according to their ability to produce acid from sugar.

I. Acid from glucose, sucrose, maltose, and lactose .....4 strains.
II. Acid from glucose, sucrose, and maltose .....22 strains.
III. Acid from glucose and sucrose..... 4 strains.
IV. Acid from glucose ..... 1 strain.

Considering the results of the acid production test, using raffinose and galactose, and of the film formation test by Stelling-Dekker with the above groups, the author presents a new classification system for *Debaryomyces* as follows:

Group I. Acid from glucose, sucrose, maltose, and lactose.

Debaryomyces tyrocola var. *Hansenii* (Zopf) Dekker
D. Guilliermondii var. *nova-zeelandicus* Lodder
D. orientalis Naganishi
D. sp. R_4

Group II. Acid from glucose, sucrose, and maltose.

a) Film-formation on wort.
b) Greyish-white folded film.

D. membranaefaciens Naganishi
D. „ var. *Zingiberi* Otani
D. „ var. *hollandicus* Lodder
D. sp. O_9 (*D. membranaefaciens*)
D. sp. C_2 (*D. membranaefaciens* type)

bb) Grey thin film.
c) Acid from raffinose.

D. miso Mogi
D. miso var. α Mogi
D. japonicus Naganishi
D. sp. C_1 (*D. tyrocola* type)
D. sp. C_4 („ „)
D. sp. S_3 („ „)
D. sp. P_2 („ „)

cc) Acid from raffinose weakly.

D. sp. Y_1 (*D. tyrocola* type)
D. sp. Q_3 („ „)

D. sp. Q_4 („ „)

aa) No film on wort.

b) Gas and acid from glucose.

c) Acid from galactose.

D. manchuricus Naganishi

cc) No acid from galactose.

D. globosus Klöcker.

bb) No gas from glucose.

c) Acid from raffinose.

D. Klöckeri Guilliermond et Péju

D. sp. S_4 (*D. Klöckeri* type)

D. sp. A_2 (*D. Matruchoti* type)

cc) No, or less acid from raffinose.

D. sp. V_1

D. sp. X_1

Group III. Acid from glucose and sucrose.

a) Acid from galactose.

b) Acid from raffinose.

D. Matruchoti

bb) No acid from raffinose.

D. sake Saito et Oda

Mycoderma sp. Q

aa) No acid from galactose.

D. sp. S_2 (*D.* n. sp. unpublished)

Group IV. Acid from glucose.

D. sp. W_1

Discussion

The routine fermentation test is not of great singnificance in the case of *Debaryomyces*, but the acid production test is worthy of consideration as an important factor in differentiating the species of this genus. In employing this test it is necessary to decide the number of culturing-days required to judge the ability of acid production. Because of the following facts, the author considers it suitable to adopt the results of six culturing days:

1) In some rapid cases, acid production was observed within 24 hours, and it could be detected within 4–6 days even in the slow acid-producers; 2) when the test culture is allowed to incubate for a prolonged period, the color change becomes obscure; 3) a few species sometimes display a tendency to revert to the initial color after once changing; 4) the color of the medium itself may change during a long incubation.

As pointed out by a few workers, intermediate strains between *D. membranaefaciens* type and *D. Guilliermondii* type are very difficult to separate by the state of the film, whereas by the acid production from lactose, both types are easily distinguishable. *D. Guilliermondii* strain K, *D.* sp. H (*D. Guilliermondii* strain ?), *D. Guilliermondii* Dekker, and some of the other strains will probably be qualified for Group I, because Naganishi's test indicated that they were capable of assimilating lactose. This assimilability was exhibited by 4 of the 13 strains representing *D. tyrocola*, though by the author's test, all strains of this type that were tested were unable to produce acid from lactose and belonged to Group II.

This system is considered to be applicable for the taxonomy of *Pichia* and *Zygopichia*, having oxidative or weak fermentative ability, since the new system, based on acld production, gave simple and distinct diagnoses of *Debaryomyces*. It will become more useful by adopting the utilization test of nitrite and urethan (Naganishi 1948), and the growth test at moderate osmotic pressure (Wickerham 1951).

Summary

A standard test detecting the acid production for taxonomic studies of *Debaryomyces* was devised, and the acid-producing ability was investigated on 31 strains.

The yeasts tested, were divided into 4 groups, according to their ability of producing acid from glucose, maltose, sucrose, and lactose. Moreover, they were classified by the acid production test with raffinose and galactose, as well as by the state of the film. Thus a new classification system of *Debaryomyces* has been proposed.

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(The mark* indicates an essay which was published in Japanese).