THE BIOCHEMICAL CLASSIFICATION OF YEAST STRAINS[1]

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The classification of yeasts has been based to some degree on their ability to ferment certain sugars. This test is one of the well-established biochemical methods. Stelling-Dekker (1931) systematically examined the genus *Saccharomyces* and reorganized its classification unequivocally upon fermentation ability. However, this system, good though it be, is inadequate for the classification of the great number of yeast strains which, because of fermentative ability, must be called either *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*. Designation of cultures as sub-species, varieties, strains and races is often based on individual characteristics which are evident in a particular process. The microbiologist is seldom able to identify exactly a fresh isolation or an unknown culture and must perforce carry it in his collection with a numerical designation. Although this inability may not be a great hindrance to industry, it is a serious problem for microbiology. The study of growth factors in particular has been greatly hindered by the lack of sharp differentiation between cultures.

As briefly reported elsewhere, Schultz, Atkin and Frey (1938–9) found a growth reaction which permits a sharp differentiation between otherwise closely related varieties of yeast. This difference appears in both the *S. cerevisiae* and *S. carlsbergensis* groups and does not appear to be related to the size or shape of cells so far as we have been able to determine.

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EXPERIMENTAL

The test is based on the growth response to vitamin B_1 and B_6. A synthetic medium is prepared, which contains an adequate amount of the bios ingredients, as developed by W. L. Miller working with the yeast culture known as *S. cerevisiae* Toronto. By adequate bios we mean enough to give growth of *S. cerevisiae* Toronto equivalent to the crop obtained from 9° Blg. malt wort under the stated conditions of temperature, time, and seeding. The bios ingredients are Bios I (inositol); Bios IIA (β-alanine); and Bios IIB, a purified preparation readily made from a number of sources by carbon absorption and elution repeated thrice. We have employed cane molasses residues. It is necessary for the present work that the eluate be only substantially free from vitamins B_1 and B_6. The synthetic medium contains, in addition to glucose and inorganic nutrient salts, a buffer of potassium citrate and citric acid, designed to approximate the buffering capacity of malt wort. We have obtained a full crop with virtually all the yeasts tested, using $(NH_4)_2SO_4$ and β-alanine. Failure to consider all the known growth factors, or perhaps use of inadequate growth standards, may have led Nielsen and Hartelius (1939) to suggest that asparagine is necessary before β-alanine can act as a growth substance.

Twenty-nine milliliters of this sterile basic medium are placed in a 200 ml. erlenmeyer flask. The ammonium salt is sterilized separately to avoid reaction with glucose. One milliliter of a suspension containing one milligram of moist yeast is now added and the flask is shaken at 30°C. for 24 hours. Two milliliters of 10 per cent chloracetic acid are placed in the bottom of a Hopkins vaccine tube and then 1 to 5 ml. of the yeast suspension added. The tube is allowed to stand for 5 minutes before water is added to the 10 ml. mark and is then centrifuged at high speed for 5 minutes. The volume of yeast corresponding to 1 ml. of the yeast suspension multiplied by 10,000 gives the crop figures which are reported. It has been separately determined that 1 gram of moist yeast suspended in a volume of 30 ml. gives a crop, by this method of calculation, of 220. Thus, a crop figure in the neighborhood of 200 represents a thousandfold increase in yeast.

The yeast used to inoculate the growth tests is grown in an analogous manner on a malt wort of 9° Blg. prepared from Fleischmann's special diastatic dry Diamalt. The pH is set at 5.0 with sulphuric acid and the whole autoclaved at 15 lbs. for 15 minutes, then filtered while hot and distributed in 30 ml. lots in 200 ml. erlenmeyer flasks. These flasks, after intermittent sterilization, are inoculated from the agar slant with a platinum needle giving a fairly good-sized inoculum (roughly 1 to 2 cubic millimeters). All of the yeasts reported gave crops between 120 and 230, with the great majority around 200. Other malt media or beer worts may be conveniently used. A portion of the yeast suspension is prepared for use in the growth test by washing twice in sterile distilled water (in ordinary 15 ml. centrifuge tubes) and then diluting so that 1 ml. of centrifuged yeast is suspended in a liter of water, i.e., approximately one milligram of moist yeast per ml.

For the purposes of the growth tests to be described 10 gamma of thiamin is added to each 30 ml. of the basal medium and 10 gamma of each of thiamin and vitamin B_6 to another portion.

The cultures used were obtained from the American Type Culture collection, the Centraalbureau voor Schimmelcultures at Delft, and the Institut für Gärungsgewerbe at Berlin. A number of cultures were identified in the American Type Culture collection as *Saccharomyces ellipsoideus*, but following Stelling-Dekker we have considered them as varieties of *S. cerevisiae* Hansen. The yeasts were cultured on malt agar.

RESULTS

Table 1 gives typical results on four *S. cerevisiae* cultures. Type A yeasts grow poorly on the basal medium and better with thiamin and still better when vitamin B_6 is added. Types B and C yeast grow less well in the presence of thiamin, but B_6 overcomes the inhibition. In our earlier publications we described only A and B type yeasts, but we have since found a few yeasts whose crops are depressed more than half by thiamin. It is felt that this difference is sufficient to justify a third type (type C). On the other hand we have extended type B to cover those yeasts which show only slight inhibition by thiamin, or no inhibition at all.

We have thus far found ten strains (table 2) of yeast of type A, carried in the collections as varieties, strains or races of *S. cerevisiae* Hansen.

TABLE 1

Example of Saccharomyces cerevisiae Hansen types A, B and C

CULTURE	SOURCE	CROP VALUES			TYPE
		Basal medium	Plus thiamin	Plus thiamin and B_6	
Strain Delft I.....	C. B. S.*	5	110	180	A
Strain Anamensis.....	C. B. S.	230	200	220	B
Strain Fulmer no. 11..	A. T. C. C. no. 4226	200	150	190	B
Strain Alpinus.....	C. B. S.	150	35	200	C

* The following abbreviations are used in this and subsequent tables: A. T. C. C., American Type Culture Collection; C. B. S., Centraalbureau voor Schimmelcultures; I. f. G., Institut für Gärungsgewerbe; R. J. W., Roger J. Williams; W. L. M., W. Lash Miller.

TABLE 2

Saccharomyces cerevisiae Hansen type A

CULTURE	SOURCE	CROP VALUES		
		Basal medium	Plus thiamin	Plus thiamin and B_6
Var. ellipsoideus strain Delft II.....	C. B. S.	12	85	180
Strain Delft I.....	C. B. S.	5	110	180
Race XII.....	I. f. G.	5	180	190
Luft II.....	I. f. G.	15	170	190
Distillery yeast.....	A. T. C. C. no. 4111	90	170	205
Bakers yeast.....	A. T. C. C. no. 2335	5	60	130
Brewers yeast.....	A. T. C. C. no. 2310	5	120	150
Distillery yeast.....	A. T. C. C. no. 4109	20	120	140
Wildiers yeast.....	R. J. W.	25	30	90
Distillers yeast.....	A. T. C. C. no. 286	20	30	80

There is no reason for confusing any of these yeasts with types B and C. In spite of definite but reproducible differences among them, all show a great increase in growth in the presence of B_1

and B_6. The double space separating groups of these yeasts may foreshadow a further subclassification of type A yeasts. Such a

TABLE 3

Saccharomyces cerevisiae Hansen type B

CULTURE	SOURCE	CROP VALUES		
		Basal medium	Plus thiamin	Plus thiamin and B_6
Str. anamensis	C. B. S.	230	200	220
Str. batatae	C. B. S.	200	210	230
Nat'l. Coll. Type Cultures no. 467	A. T. C. C. no. 2338	210	190	190
Tokay wine yeast	A. T. C. C. no. 4108	180	170	180
"Magne" distillers	A. T. C. C. no. 4132	200	190	200
Distillers	A. T. C. C. no. 4124	210	195	205
St. George wine	A. T. C. C. no. 4118	200	170	190
California wine	A. T. C. C. no. 4105	190	160	180
Wild yeast (American)	A. T. C. C. no. 4127	200	190	205
Race M	I. f. G.	205	160	220
Spc. 152	I. f. G.	210	160	220
Toronto strain	W. L. M.	205	170	200
Strain Fulmer no. 11	A. T. C. C. no. 4226	200	150	190
Sulphite Yeast	A. T. C. C. no. 765	220	150	210
McDermott no. 74	A. T. C. C. no. 4100	200	130	190
Menes wine yeast	A. T. C. C. no. 4117	200	150	190
German wine yeast	A. T. C. C. no. 4129	200	130	180
Distillers yeast	A. T. C. C. no. 4125	180	130	200
Jordan wine yeast	A. T. C. C. no. 288	190	140	185
Laviero wine yeast	A. T. C. C. no. 4114	190	130	185
Hungarian beer yeast	A. T. C. C. no. 764	190	130	190
French wine yeast	A. T. C. C. no. 4921	170	100	190
Distillers (Amer. grain)	A. T. C. C. no. 4110	140	120	205
French wine yeast	A. T. C. C. no. 4116	100	75	170
Ansmushausen wine	A. T. C. C. no. 4113	100	50	120
Pribam collection	A. T. C. C. no. 2368	35	15	65

subclassification might readily be established even now but it is felt that it is not necessary at the present. The fifth yeast has a higher-than-usual crop on the basal medium, whereas the last

five yeasts have lower crops on the medium containing both B_1 and B_6.

Of type B yeasts we have found 26 (table 3). Closer examination discloses that many are probably identical, but as mentioned before they have found their way into separate test tubes and no one would dare to mix them. As is apparent, these yeasts do not seem to require either thiamin or vitamin B_6. The first nine differ from the rest in so far as the depression of crop due to thiamin is at a minimum. The next thirteen are characterized by a somewhat greater drop in crop when thiamin alone is added.

The following three are similar to the first twenty-two but are characterized by a low crop on the basal medium. The last

TABLE 4

Types of Saccharomyces carlsbergensis

CULTURE	SOURCE	CROP VALUES			TYPE
		Basal medium	Plus thiamin	Plus thiamin and B_6	
Var. valdensis	C. B. S.	25	40	55	A
Strain Frohberg	C. B. S.	20	35	48	A
Chubut	I. f. G.	220	180	220	B
Var. mandshuricus I	C. B. S.	100	25	200	C
Var. polymorphous	C. B. S.	120	40	220	C
Kopenhagen	I. f. G.	Nil	Nil	Nil	
A. T. C. C. no. 2345	A. T. C. C.	Nil	Trace	Trace	

yeast is quite typical of B type yeasts, but all crops are low. As mentioned above, sub-types might readily be established on the basis of the differences shown, but we would like first to locate the deficiency made evident by the growth of such yeasts as the last one.

The type C yeast shown in table 1 is the only one which we have found under *S. cerevisiae.*

The results with seven strains of *S. carlsbergensis* are shown in table 4. The first two appear to belong to type A, although the low crops make them somewhat atypical. There is no question, however, that they differ from the other *S. carlsbergensis* strains. The third yeast is clearly a type B, and the next two are type C.

The last two *S. carlsbergensis* strains are in a class by themselves, inasmuch as the best medium is quite inadequate for them.

DISCUSSION AND CONCLUSIONS

The reaction of 37 strains of *S. cerevisiae* Hansen to the test described permits a definite and unequivocal subclassification without recourse to morphology. It is therefore suggested that cultures of *S. cerevisiae* be designated as *S. cerevisiae* Hansen Type A, etc. *S. carlsbergensis* might be treated in an analogous manner. Once the type has been determined, one may have recourse to morphological differences unless, as appears likely, further bios studies permit further biochemical classification. Recently Rainbow (1939) described a biochemical classification of yeasts based on bios tests. Unfortunately he was unable to repeat our observations with vitamin B_1 and B_6. His failure may have been due to the employment of a crude bios IIA preparation whereas we employ β-alanine. One may expect that other problems of classification in microbiology will be attacked and perhaps solved by the careful study of growth requirements and reactions.

SUMMARY

Examination of 44 yeast cultures described as strains, varieties or races of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* has shown that they may be further divided into:

Type A: yeasts which give a crop that is low on the basal medium, is increased by thiamin and further increased by vitamin B_6.

Type B: yeasts which give a crop that is high on the basal medium, is depressed by thiamin (not more than 50 per cent), and is normal in the presence of thiamin and B_6.

Type C: yeasts which give a crop that is depressed more than half by thiamin but which give a high crop on addition of both thiamin and B_6.

A few cultures are described which, because of low crops on all media, cannot be typed with certainty.

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