[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY OF OREGON]

THE FURTHER FRACTIONATION OF YEAST NUTRILITES AND THEIR RELATIONSHIP TO VITAMIN B AND WILDIERS' "BIOS"

BY ROGER J. WILLIAMS AND ELIZABETH M. BRADWAY

RECEIVED DECEMBER 8, 1930 PUBLISHED FEBRUARY 9, 1931

Different so-called strains of *Saccharomyces cerevisiae* have diverse requirements for their growth stimulation as is evidenced by the contradictory results of many workers[1] and the experimental study made in this Laboratory.[2] In this publication it was reported that yeast of the type "Gebrüde Mayer" (wholly unlike several others) is stimulated almost as effectively by extracts which have been shaken with fuller's earth as by untreated extracts. Similar results had been obtained previously on yeast autolysate by Eddy, Kerr and Williams,[3] when they cultured "Gebrüde Mayer" yeast.

We have tried numerous means to purify this yeast nutrilite which is not adsorbed by fuller's earth, but with only moderate success. No adsorbent has been found which will remove it from solution effectively and not one of the numerous precipitants tried, precipitates the substance in question satisfactorily. The most potent preparations obtained by us were made by using various adsorbents and precipitants in sequence and in each case rejecting the precipitated or adsorbed material. We have thus obtained material approximately ten times as potent as the original material which was extracted directly from rice polish. Experimental details regarding this work will not be presented at this time because we feel that our efforts have been rewarded with comparatively little success and it will be better to wait until we have made further progress on the problem.

On several occasions we tested preparations to ascertain whether we had separated two factors which might be ineffective when introduced singly but which might supplement each other. We have no indications that this is the case and so far as present evidence is concerned the yeast nutrilite which stimulates the growth of Gebrüde Mayer yeast may be a single substance. Our results on this are in agreement with those of Eddy and his co-workers[3,4] who have worked with Gebrüde Mayer yeast and have never reported a supplementary action between their preparations. Peskett and O'Brien,[5] who are probably working with this same (baker's)

[1] It is not appreciated by all investigators in this field that commercial yeast propagation is not necessarily a fixed and immutable process, and that improved strains of yeast may be introduced from time to time. Hence the term "bakers' yeast," for example, does not have a definite meaning when used in connection with these studies.

[2] R. J. Williams, M. E. Warner and R. R. Roehm, THIS JOURNAL, **51**, 2764 (1929).

[3] W. H. Eddy, R. W. Kerr and R. R. Williams, *ibid.*, **46**, 2846 (1924).

[4] R. W. Kerr, *Proc. Soc. Expt. Biol. Med.*, **25**, 344 (1928).

[5] G. W. Peskett and J. R. O'Brien, *Chemistry Industry*, June 20, 1930.

yeast, have found no evidence that their "bios" is a complex. Narayanan,[6] who has studied a yeast presumably of the same type, says "No evidence to support the complex nature of 'bios' has been obtained."

The description of "bios" originally given by Wildiers was that of a substance which was not precipitated by any of the ordinary precipitants. In fact its properties seemed to be very much like those of the nutrilite which stimulates Gebrüde Mayer yeast. We thought it worth while to study further the question as to the identity of the Gebrüde Mayer nutrilite and the "bios" of Wildiers. For this purpose we were fortunate in obtaining from Professor M. Ide of Louvain, Belgium, a culture of the yeast with which Wildiers' original work was done. The yeast culture as received was labeled *Strain of Sacch. Cerev. 1 Hansen, Saccharomyces Wildiersii.* For convenience we shall refer to this as Wildiers' yeast.

Wildiers' yeast, like Gebrüde Mayer, is stimulated nearly as well by yeast extracts which have been treated with fullers' earth as by untreated extracts. This is shown by the results given in Table I. The tests were carried out and the determinations of yeast crops made in accordance with the technique outlined in previous publications[2,7,8] from this Laboratory. The yeast extract was prepared by extracting dry yeast with eight times its weight of hot 60% methanol, evaporating the filtrate to dryness and making up to one-half of its original volume with water. Its P_H was adjusted to about 5, and a 50-cc. portion was shaken for ten minutes with 2.5 g. of fuller's earth and filtered.

TABLE I

TREATMENT OF EXTRACTS WITH FULLER'S EARTH (WILDIERS' YEAST)

Addition to synthetic medium (10 cc.)		Galvanometer deflection	Yeast crop, mg., dry wt.
1 cc. H_2O		34.5	0.43
1 cc. H_2O		35.45	.38
Untreated extracts from	5 mg. of yeast	32.8	.58
	5 mg. of yeast	33.35	.54
	10 mg. of yeast	29.6	.91
	10 mg. of yeast	30.65	.81
	20 mg. of yeast	25.2	1.38
	20 mg. of yeast	24.55	1.47
Same extracts treated with fuller's earth	5 mg. of yeast	32.35	0.64
	5 mg. of yeast	33.3	.54
	10 mg. of yeast	31.2	.74
	10 mg. of yeast	31.0	.75
	20 mg. of yeast	27.45	1.14
	20 mg. of yeast	27.4	1.13

Original total seeding about 1,400,000 cells.

[6] Narayanan, *Biochem. J.*, **24**, 6 (1930).

[7] R. J. Williams and R. R. Roehm, *J. Biol. Chem.*, **87**, 581 (1930).

[8] R. J. Williams, E. D. McAlister and R. R. Boehm, *ibid.*, **83**, 315 (1929).

In this and other experiments which are not reported in detail, some activity is removed by fuller's earth treatment and by the use of a sufficient amount of fuller's earth a considerable amount of activity may be removed. However, we have no evidence that the nutrilite which functions for Wildiers' yeast is any different from that which stimulates the growth of Gebrüde Mayer yeast. While we have had some slight indications in this direction, we must say that we have no proof either from our work or that of others that Wildiers' "bios" is other than a single substance. While preparations made in different ways aid in the stimulation of this yeast, evidence as to a supplementary relationship between them is largely lacking. Later experiments may prove that Wildiers' "bios" is multiple in nature.

We have what seems to be conclusive evidence that the yeast growth stimulants, with which W. Lash Miller[9,10] and his associates in Toronto have been working, are entirely different from Wildiers' "bios." Through the kindness of Dr. Miller we have been furnished with a culture of the yeast with which his experiments have been carried out.

We have been able to show that unlike any other of the seven representative yeasts tested in our laboratory, W. L. Miller's yeast is stimulated in its growth to an appreciable extent by highly purified inositol. In this respect Miller's yeast behaves entirely unlike Wildiers' yeast, as is shown in Table II. This is one of two separate experiments which gave similar results. It will be noted that our method of testing shows a very definite response on the part of Miller's yeast to inositol alone when as much as 1 mg. is added to 12 cc. of medium. This response is definitely lacking in the case of Wildiers' yeast.

TABLE II

EFFECT OF INOSITOL ON W. L. MILLER'S AND WILDIERS' YEASTS

Addition to synthetic medium	W. L. Miller's yeast Galv. reading	W. L. Miller's yeast Mg. of yeast	Wildiers' yeast Galv. reading	Wildiers' yeast Mg. of yeast
1 cc. of H_2O	31.25	0.74	35.35	0.38
1 cc. of H_2O	31.85	.69	36.6	.27
1 mg. of inositol in 1 cc. of H_2O	23.7	1.59	36.7	.27
1 mg. of inositol in 1 cc. of H_2O	24.05	1.55	36.9	.26
Seeding, cells	1,300,000		1,600,000	

Even more striking evidence that the nutrilites for Miller's yeast are quite different from Wildiers' "bios" is based upon the effects of tea extracts on these two yeasts. We find, in confirmation of the work of Miller and Eastcott, that tea is a good source of the nutrilites for Miller's yeast. For this purpose our sample of tea was about the equivalent of our rice polish. However, for Wildiers' yeast and all of the other yeasts which we

[9] W. L. Miller, *Science*, 59, 197 (1924).

[10] E. V. Eastcott, *J. Phys. Chem.*, 32, 1094 (1928).

have tested, the rice polish was much richer than the tea. The contrast between these two materials is especially striking in the case of Wildiers' yeast, as is shown in Table III. In one experiment which is not reported in detail tea was distinctly superior to rice polish as a source of nutrilites for Miller's yeast. It seems fair to conclude that the growth-stimulating substances which are abundant in tea are quite distinct from Wildiers' "bios," since they do not stimulate Wildiers' yeast. This conclusion is in line with the fact that Miller and his co-workers have repeatedly and without difficulty fractionated their material into supplementary fractions, whereas attempts on our part to do so with the nutrilite which is effective for Wildiers' yeast have failed repeatedly to yield any such results.

TABLE III

EFFECTS OF RICE POLISH AND TEA EXTRACTS ON W. L. MILLER'S AND WILDIERS' YEASTS

Addition to synthetic medium	Yeast crop W. L. Miller's yeast Galv. deflection	Mg. of dry yeast	Δ over blank	Yeast crop Wildiers' yeast Galv. deflection	Mg. of dry yeast	Δ over blank
1 cc. of H_2O	31.05	0.77	..	34.8	0.40	..
Ext. of 5 mg. of rice polish	23.55	1.60	0.83	29.2	0.95	0.45
Ext. of 10 mg. of rice polish	19.15	2.22	1.45	23.95	1.56	1.16
Ext. of 5 mg. of tea	23.5	1.61	0.84	34.45	0.44	0.04
Ext. of 10 mg. of tea	20.7	1.99	1.22	33.65	.51	.11
Seeding	1,200,000 cells			1,550,000 cells		

It appears, therefore, that Miller and his associates should be credited with the discovery of a new series of yeast nutrilites, at least one of which is abundant in tea. These appear to be entirely distinct so far as present evidence indicates from the "bios" described by Wildiers.

The requirements (for growth stimulation) of old process bakers' yeast (with which the senior author's first experiments were carried out), yeast 578 of the American Type Culture collection and *Untergärige Hefe K*, seem to be more complex than those of Wildiers' yeast. They can readily be resolved into a number of interdependent growth stimulants of which the "bios" of Wildiers may be one, since one of the factors appears to have properties and occurrences similar to those of Wildiers' "bios."

The growth stimulants for the yeasts listed above appear to be different from those concerned in the growth stimulation of Miller's yeast, since these yeasts are not affected appreciably by inositol and we have been unsuccessful in fractionating the nutrilites by the same means used in the Toronto laboratories.

Our work on these yeasts has recently been concerned largely with yeast 578, partly because a considerable amount of work was done with it when the old process culture was not available and partly because it seems to give relatively regular and reproducible results.

Previous work published in 1927[11] has shown that yeast 578 requires for growth stimulation two substances (or groups of substances) which can be separated by adsorption of fuller's earth and which have little effect when introduced into the medium singly. More recently[12] it was shown that the crystalline antineuritic vitamin as prepared by Jansen and Donath is extremely potent as a yeast growth stimulant when used in conjunction with the residue of a yeast extract which is not adsorbed by fuller's earth. It was also shown, however, that the antineuritic vitamin of Jansen and Donath is not the only substance which can function in this manner.

Previous publications from this Laboratory have shown, therefore, that for the stimulation of the growth of yeast 578 (and old process yeast) there is required (1) material not adsorbed from yeast extract by fuller's earth *plus* (2) the antineuritic vitamin of Jansen and Donath *or* (3) other material which has many precipitation reactions in common with the Jansen and Donath vitamin but is not identical with it. In other words, at least three distinct and separable nutrilites may be involved in the growth stimulation of certain yeasts.

We shall now present evidence to show the existence of a fourth separable nutrilite which is concerned in the stimulation of the growth of yeast 578.

A highly concentrated preparation which was obtained from rice polish extract by adsorption on fuller's earth, precipitation with phosphotungstic acid, precipitation with silver nitrate and baryta, precipitation with chloroplatinic acid in absolute alcohol, using the method of Jansen and Donath, we shall for convenience designate as "A." It had a potency determined in accordance with a previous publication[12] of 125–200. A preparation of material from rice polish extract which was adsorbed by and extracted from fullers' earth, we shall designate as F. E. S. (fullers' earth solids). It has a potency of 2.5–3.

As a supplement to material which is not adsorbed from yeast extract by fuller's earth (U. R. Y.), "A" is (in accordance with their potencies) about 60 times as potent as "F. E. S." However, as a supplement to unadsorbed material from rice polish extract (designated as U. R. R.), "A" was shown to be only 20 times as potent as "F. E. S." This decided discrepancy which was obtained more than once led us to suspect that a new factor was present in the unadsorbed residue from yeast extract and in the "F. E. S.," but not to an appreciable extent in the unadsorbed residue from the rice polish extract.

The suspicions were confirmed when we prepared from the fuller's earth solid a preparation "F. E. $S._5$" which supplements "A" plus "U. R. R." F. E. $S._5$ was obtained by precipitating some of the fuller's earth solids with phosphotungstic acid in the presence of sulfuric acid and by subsequent removal of the excess acids and evaporation of the *filtrate*. Simultaneously

[11] R. J. Williams, J. L. Wilson and F. H. Von der Ahe, THIS JOURNAL, **49**, 227 (1927).

[12] R. J. Williams and R. R. Roehm, *J. Biol. Chem.*, **87**, 581 (1930).

we decomposed the precipitated material with baryta and freed it from excess baryta. The precipitated material in this experiment is designated as "F. E. $S._6$"

The behavior of the "F. E. $S._5$" preparation in supplementing either "A" plus "U. R. R." or "F. E. $S._6$" plus "U. R. R." is shown in Tables IV and V. The results obtained in these experiments were in each case confirmed by separate experiments which are not reported in detail.

TABLE IV

SUPPLEMENTARY ACTION OF "F. E. $S._5$" ON "U. R. R." PLUS "A" (YEAST 578)

Addition to synthetic medium	Yeast crop: Galv. deflection	Yeast crop: Mg. of dry yeast
1 cc. of H_2O	37.65	0.26
8 mg. of "U. R. R." (in 1 cc. of H_2O)	31.65	.68
8 mg. of "U. R. R." + 0.005 mg. "A"	27.55	1.0
8 mg. of "U. R. R." + 0.2 mg. "F. E. $S._5$"	29.8	0.87
8 mg. of "U. R. R." + 0.005 mg. "A" + 0.2 mg. "F. E. $S._5$"	16.75	2.70

Seeding about 950,000 cells.

TABLE V

SUPPLEMENTARY ACTION OF "F. E. $S._5$" ON "U. R. R." PLUS "F. E. $S._6$" (YEAST 578)

Addition to synthetic medium	Yeast crop: Galv. deflection	Yeast crop: Mg. of dry yeast
1 cc. of H_2O	35.3	0.36
8 mg. of "U. R. R." (in 1 cc. of H_2O)	26.3	1.26
8 mg. of "U. R. R." + 0.1 mg. "F. E. $S._5$"	25.1	1.38
8 mg. of "U. R. R." + 0.1 mg. "F. E. $S._6$"	10.8	4.07
8 mg. of "U. R. R." + 0.1 mg. "F. E. $S._5$" + 0.1 mg. "F. E. $S._6$"	7.5	5.37
8 mg. of "U. R. R." + 0.2 mg. "F. E. $S._5$"	25.25	1.37
8 mg. of "U. R. R." + 0.2 mg. "F. E. $S._6$"	9.55	4.52
8 mg. of "U. R. R." + 0.2 mg. "F. E. $S._5$" + 0.2 mg. "F. E. $S._6$"	4.4	8.05

Seeding about 1,360,000 cells.

It will be noted in both cases that the addition of "F. E. $S._5$" alone to "U. R. R." has very little effect but that its addition to (U. R. R. + F. E. $S._6$) or (U. R. R. + A) has a very appreciable effect. Presumably the preparation "F. E. $S._5$" contains the new factor relatively free from other known factors, but "F. E. $S._6$" and other preparations probably contain some of the new factor and thus prevent the results from being as clear cut as they would otherwise be.

What the character of this new factor is or what relationship it may have to some of the components of vitamin B is largely conjecture. In view of the fact that the antineuritic vitamin may function as a yeast growth stimulant, it is entirely probable that other yeast growth stimulants of a similar nature may have some function in animal nutrition. It is certain

that the study of yeast nutrilites and attempts to isolate them are not interesting alone from the standpoint of the yeast itself but also from the standpoint of higher organisms, since substances which stimulate yeast growth are present in practically all plant and animal tissues and may be presumed to have some function in these tissues.

We wish to acknowledge gratefully the support received for this research from the Research Council of the University of Oregon and from Standard Brands, Inc., Successors to the Fleischmann Company, etc.

Summary

1. Experiments with Wildiers' original yeast culture shows that his "bios" is not readily absorbed by fuller's earth and appears to be the same as the nutrilite which stimulates the growth of Gebrüde Mayer yeast. No conclusive evidence of its multiple nature is available.

2. The yeast nutrilites studied by W. L. Miller and his associates are shown to be distinct from the "bios" of Wildiers. The Toronto workers should be credited with the discovery of a new series of yeast nutrilites.

3. Yeast No. 578 of the American Type Culture Collection and old process baker's yeast appear to have more complex requirements than Wildiers' yeast. Evidence is presented for the existence of a fourth distinct nutrilite concerned in the growth stimulation of yeast No. 578. It seems reasonable to suspect that several of the nutrilites for this yeast may be components of "vitamin B," since the antineuritic vitamin has previously been shown to affect its growth very strikingly.

EUGENE, OREGON

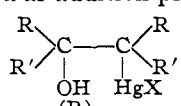
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

THE STRUCTURE OF THE COMPOUNDS PRODUCED BY THE ADDITION OF MERCURIC SALTS TO OLEFINS. II

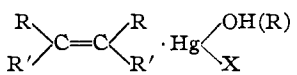
BY ESTHER GRIFFITH AND C. S. MARVEL

RECEIVED DECEMBER 15, 1930 PUBLISHED FEBRUARY 9, 1931

Some time ago a communication[1] from this Laboratory described optically active isomers of an addition product of an olefin of the type RR'C=CRR' with a mercuric salt. The conclusion was drawn that the existence of these isomers furnished evidence for believing that such addition products should be represented by ordinary structural formulas (I) rather than as molecular addition products (II).



I



II

[1] Sandborn with Marvel, THIS JOURNAL, **48**, 1409 (1926).