[CONTRIBUTION FROM FOREST PRODUCTS LABORATORY,[1] FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Acclimatization of Various Yeasts to Wood Sugar[2]

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Wood hydrolyzates are rapidly gaining recognition as sources of sugar for the production of both ethyl alcohol and a high protein yeast. The pilot-plant production of alcohol by *S. cerevisiae*[1,2] and *Torula utilis* No. 3[3] has been described previously, as has the propagation of *Torula utilis* No. 3 on wood sugars.[4,5,6,7]

Although the production of alcohol and yeast from wood hydrolyzates has been practiced for a number of years in America[8,9,10] and Germany,[11,12,13] recent pilot-plant work has indicated that difficulties arise when conventional methods are used. The presence of inhibiting substances from either the wood or the corrosion products from the equipment retards the development of yeast previously grown exclusively on a blackstrap molasses or glucose substrate. Wood-sugar hydrolyzates are sterile because of the drastic conditions used for their production and remain so because of the inhibiting substances.

For industrial use it is recognized that the yeast must have the following characteristics: (1) the ability to propagate readily in the substrate and be easily propagated in large quantities; (2) the ability to maintain a uniformity of growth or rate of fermentation; and (3) the ability to grow and ferment without the use of special treatments for the substrate or the addition of substances other than the inorganic salts needed to supply the nitrogen, phosphorus and potash required for yeast propagation.

The successful acclimatization of *Torula utilis* No. 3, a fodder yeast, after repeated transfers for the production of alcohol, in high yields from wood sugar, has suggested the possibility of adapting other yeasts to wood hydrolyzates. This report describes an investigation made at the U. S. Forest Products Laboratory of both the propagation of yeast and the production of alcohol. Twenty strains of yeast were tested plus the strain *Torula utilis* No. 3, which was used for comparison.

Yeast Growth on Wood Hydrolyzate.—To determine the ability of the various yeasts to propagate in approximately 5% wood-sugar solutions, tests were made with the undiluted hydrolyzate, which had been produced by passing dilute sulfuric acid through a charge of wood at elevated pressure, neutralizing with lime and filtering the resulting solution. Except for adjusting the *p*H to 5.4 and adding urea to produce 0.10% solution and potassium dihydrogen phosphate to produce 0.05%, no other treatment was given the solution, because it was believed that pretreatments should be avoided if possible.

The yeasts were prepared by transferring a small amount of each yeast from a glucose–malt sprout agar slant into 30 ml. of glucose-malt sprout liquid medium in 8-inch Pyrex tubes. The cultures were placed in a shaker and incubated at 30° for twenty-four hours, after which time the contents of each tube were poured into 150 ml. of the liquid glucose medium in 500-ml. Erlenmeyer flasks. The cultures were again shaken for twenty-four hours at 30°.

Yeast sufficient to make an inoculum of 1.0 wet-volume per cent. was resuspended in 200 ml. of the wood sugar medium in 1-liter Erlenmeyer flasks. To allow admission of the large amounts of air required for growth, cotton plugs were inserted in the flasks and the inoculated medium was incubated at 30° in a shaker, which had a stroke of 10 cm. and was operated at 90 cycles per minute. After twenty-four hours all the yeast from each flask was removed by centrifuging from the sugar solution and resuspended in fresh wood-sugar media and the procedure repeated. Subsequently transfers were made each twenty-four hours until the yeast had utilized 80% or more of the sugar in solution. At this time the yeast population had increased about tenfold, after which only sufficient yeast to make a 1.0 wet volume per cent. yeast was transferred each twenty-four hours. Figure 1 illustrates the growth of yeast and sugar utilization with *Torula utilis major*. Other yeasts behaved in similar manner, some showing greater and others less inhibition in the 5% wood-sugar solution.

Aseptic conditions were not used after the yeast was added to the wood-sugar solutions, but extreme care was used to avoid the possibility of mixing strains. Periodic checks indicated that the

(1) Maintained at Madison, Wis., in coöperation with the University of Wisconsin.

(2) Presented at American Chemical Society meeting, Chicago, April, 1948.

(3) R. H. Leonard and G. J. Hajny, *Ind. Eng. Chem.*, **37**, 390 (1945).

(4) E. E. Harris, G. J. Hajny, Martha Hannan and S. C. Rogers, *Ind. Eng. Chem.*, **38**, 896 (1946).

(5) E. E. Harris, Martha Hannan, R. R. Marquardt and Janet T. Bubl, presented before the Sugar Division of American Chemical Society, September, 1946.

(6) W. H. Peterson, J. F. Snell and W. C. Frazer, *Ind. Eng. Chem.*, **37**, 30 (1945).

(7) E. E. Harris, J. F. Saeman, R. R. Marquardt, Martha Hannan and S. C. Rogers, presented before the Sugar Division of American Chemical Society, Chicago, Ill., September, 1946.

(8) E. E. Harris, Martha Hannan and R. R. Marquardt, *Paper Trade J.*, **125**, 34–37 (1947).

(9) E. E. Harris, Martha Hannan and R. R. Marquardt, presented at spring meeting of American Chemical Society, Atlantic City, N. J., April, 1947.

(10) F. W. Kressman, U. S. Dept. Agric., Bulletin 980 (1922), "Manufacture of Ethyl Alcohol from Wood Waste."

(11) J. F. Saeman, E. G. Locke and G. K. Dickerman, FIAT Report 499 (1945).

(12) F. K. Skoog, PB Report 2041 (1946).

(13) P. L. Pavcek, TIIC Reports (1945).

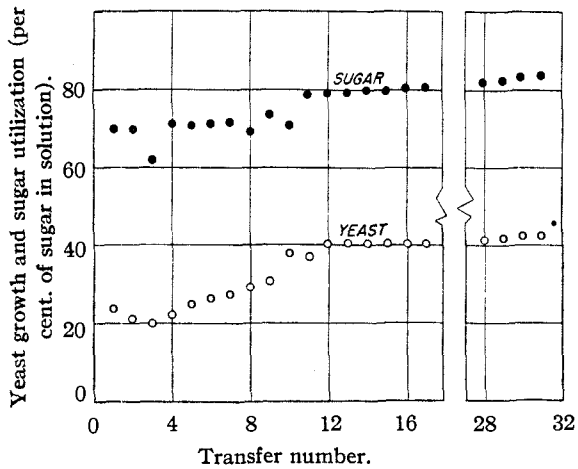

Fig. 1.—Growth of yeast and sugar utilization with *Torula utilis major* on 5% Douglas-fir hydrolyzate transferred once each twenty-four hours.

solutions remained uncontaminated and that there was no mixing of strains.

On various occasions additional samples were prepared and transferred only after forty-eight, seventy-two or ninety-six hours in order to determine if a higher yield of yeast could be obtained and if longer periods in contact with the solution affected subsequent results. Apparently, if the yeast is allowed to remain in the solution longer than the time necessary for the complete utilization of the sugar, autolysis of many of the yeast cells occurs and the activity of the remaining cells is inhibited, as indicated by incomplete sugar utilization and low yeast production for the two or three transfers following the longer periods in contact with the solution. This effect is proportional to the length of time the cells are in contact with the spent solutions. Recovery of activity was difficult after ninety-two hours of fermentation.

The yeast was grown for thirty consecutive transfers on 5% wood-sugar solutions. Comparative data regarding the growth of the various strains of yeast at the first and twelfth transfers are given in Table I. The results indicate that there is a good possibility of acclimatizing several of the strains to a medium, which in the first transfer was inhibitory. In spite of the fact that the cells became coated or permeated with a dark-colored material, they were able to overcome the inhibitive effects and remained active throughout the investigation.

Acclimatization of Yeast for Alcoholic Fermentation.—The medium used to test the ability of the strains of yeast to convert wood sugar to ethyl alcohol was the same as that used for yeast propagation, except that the *p*H was adjusted to 5.8. The culturing and shaking technique employed was similar, except that air was excluded from the flasks by inserting Bunsen valves in place of the cotton plugs. The entire amount of yeast from each flask was carried

TABLE I

GROWTH OF VARIOUS STRAINS OF YEAST IN WOOD HYDROLYZATE IN TWENTY-FOUR HOURS

Strain	Initial sugar utilized,[a] %		Yield of yeast,[a] %	
	First transfer	Twelfth to fifteenth transfer	First transfer	Twelfth transfer
Torula utilis major	78	82	25	42
Torula utilis thermophilus	78	80	33	38
Torula utilis No. 2	78	82	25	38
Torula utilis No. 900	47	79	20	38
Torula utilis No. 3	50	88	22	37
Torula utilis No. 660	80	83	32	35
Torula utilis No. 793	80	85	31	38
Torula utilis No. 957	80	82	30	37
Candida albicans	81	94	32	41
Candida arborae	45	86	21	35
Candida arborae No. 197	30	83	15	37
Candida arborae No. 198	25	84	12	37
P-13	10	78	6	35
Mycotorula lipolytica	10	84	5	36
Unidentified (x)	75	83	30	35
Hansenula anamala	50	80	21	33
Hansenula suaveolens	25	82	11	34
Saccharomyces anonensis	75	84	30	36
Saccharomyces cerevisiae No. 46	50	83	22	30
Saccharomyces ellipsoideus	60	80	27	35
Best yeast No. 2	33	80	15	38

[a] Based on total sugar in solution.

through thirty transfers at twenty-four-hour intervals.

During the fermentation the initial 1% inoculum with which the fermentations were started increased to a peak at about the twelfth transfer, and this was maintained throughout the remaining series of transfers. More than half of the cells were small and discolored but remained active. The increase in sugar utilization and alcohol production for the first twelve days and the maintenance of a high level of alcohol production for the yeast strain *Torula utilis major* is shown in Fig. 2. The results of the investigation of the ability of 20 strains of yeast, after acclimatization, to produce alcohol from 5% wood hydrolyzates are shown in Table II. *Torula utilis* No. 3, which previous work had shown to be suitable, was used for comparison. Most strains of yeast that produced very low conversions during the first few transfers developed the ability to produce alcohol in twelve transfers. Best yeast No. 2, a baking yeast, was somewhat slower in its development but had ability to produce alcohol comparable with other strains at the twenty-seventh transfer.

Parallel transfers, which were allowed to stand in contact with the fermented liquor for seventy-two or ninety-six hours, showed a slightly greater conversion to alcohol, but subsequent transfers showed reduced ability to ferment wood-sugar solutions.

In this work, the yield of alcohol based on total

TABLE II

ACCLIMATIZATION OF VARIOUS STRAINS OF YEAST FOR ALCOHOL PRODUCTION IN WOOD HYDROLYZATE IN TWENTY-FOUR HOURS

Strain	Initial sugar utilized,[a] % First transfer	Initial sugar utilized,[a] % Twelfth transfer	Alcohol production,[a] % First transfer	Alcohol production,[a] % Twelfth transfer
Torula utilis major	70	84	18	37.3
Torula utilis thermophilis	60	82	18	35.4
Torula utilis No. 2	64	75	18	31.4
Torula utilis No. 900	50	80	13	36.3
Torula utilis No. 3	47	85	10	39.6
Torula utilis No. 660	74	82	33	35.4
Torula utilis No. 793	71	80	33	33.5
Torula utilis No. 957	74	79	34	36.3
Candida albicans	72	80	32	36.9
Candida arborae	74	79	34	35.1
Candida arborae No. 197	64	81	24	34.0
Candida arborae No. 198	66	82	25	33.6
P-13	9	80	0	36.3
Mycotorula lipolytica No. 1094	5	77	1	34.9
Unidentified (x)	55	80	21	36.4
Hansenula anomala	17	81	1	37.3
Hansenula suaveolen No. 838	5	80	1	37.5
Saccharomyces ananensis	53	78	20	36.3
Saccharomyces cerevisiae No. 46	27	81	10	32.0
Saccharomyces ellipsoideus	33	81	23	37.5
Best yeast No. 2	29	84[b]	1	37.9[b]

[a] Based on total sugar in solution. [b] Twenty-seventh transfer.

sugar did not equal that consistently obtained commercially with brewer's yeast, but the more promising strains gave yields comparable with brewer's yeast when used under similar conditions. In most cases the yeast grew more readily and maintained a higher resistance to the inhibiting substances in the wood hydrolyzate than was exhibited by the strains of brewer's yeast previously tested, which, when used in larger volumes,[2]

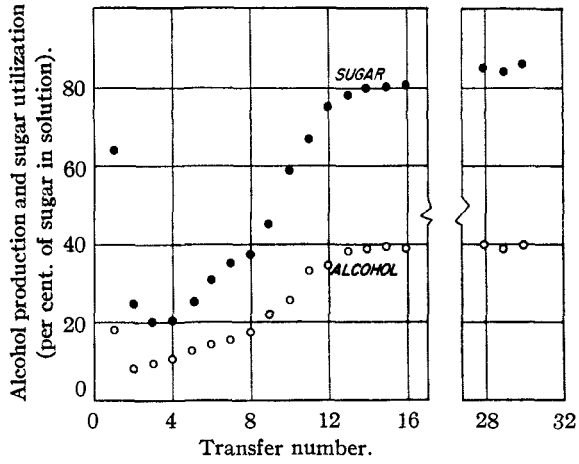

Fig. 2.—Alcohol production with *Torula utilis major* on 5% Douglas-fir hydrolyzate transferred once each twenty-four hours.

gave 39 to 40% yields of alcohol based on total sugar.

The results of these fermentations compare favorably with those obtained previously[3] with *Torula utilis* No. 3, and are shown in Table III.

TABLE III

FERMENTATION OF WOOD SUGAR BY *Torula utilis* No. 3

Fermentation volume	Average sugar utilization,[a] %	Average alcohol yield,[a] %
150 ml. in 1-liter flask	80	34.5
7 liters in 10-liter bottle	82	38.6
50 gallons in 65-gallon tank	82	40.0
350 gallons in 500-gallon tank	78	40.1

[a] Based on total sugar in solution.

Conclusion

An investigation of the ability of twenty strains of yeast to be acclimatized for yeast propagation and alcohol fermentation on untreated 5% wood hydrolyzate showed that many strains of yeast can be adapted for such purposes.

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