Table IV. Effect of Shaking and Heating Time on Recovery of DDA from Urine Using Final Method

Shaking Time, Hours	DDA, P.P.M.	Heating Time, Hours	DDA, P.P.M.
0.5	5.33	0.5	5.14
1	5.29	1	5.18
2	5.20	2	5.09
3	5.33	3	5.02
Mean	5.29		5.11
Std. deviation	0.0613		0.0689
% variation	1.2		1.3

form of Amberlite IRA-400 has recently been discontinued. However, the chloride form is satisfactory and may be substituted for the hydroxyl form without any special treatment.

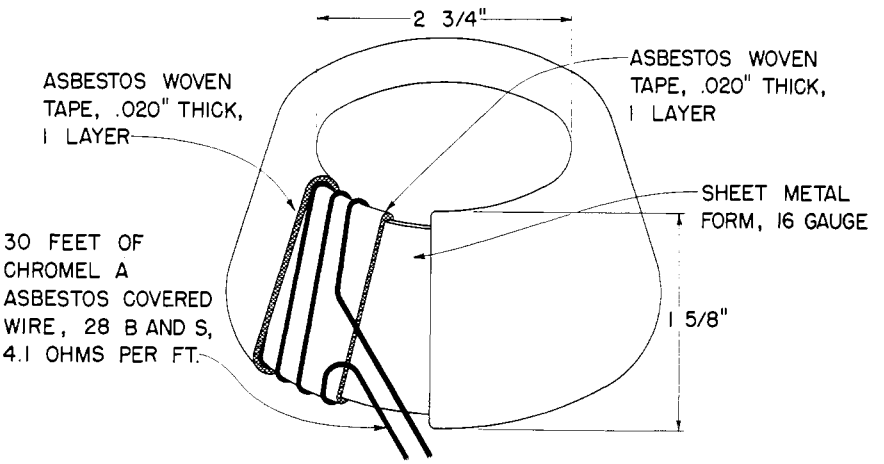

Figure 1. Flask heater for determination of DDA in urine

Literature Cited

(1) Communicable Disease Center, Technical Development Laboratories, U. S. Public Health Service, Savannah, Ga., Chemical Memorandum No. 1, 1st revision, 1953.

(2) Neal, P. A., Sweeney, T. R., Spicer, S. S., von Oettingen, W. F., *Pub. Health Repts. (U. S.)* **61,** 403 (1946).

(3) Ofner, R. B., Calvery, H. O., *J. Pharmacol. Exptl. Therap.* **85,** 363–70 (1945).

(4) Schechter, M. S., Soloway, S. B., Hayes, R. A., Haller, H. L., *Ind. Eng. Chem., Anal. Ed.* **17,** 704–9 (1945).

(5) White, W. C., Sweeney, T. R., *Pub. Health Repts. (U. S.)* **60,** 66 (1945).

Received for review December 3, 1955. Accepted June 22, 1956.

BACTERIAL CONTAMINANTS

Effect of Brewer's Yeast Strain on *Flavobacterium proteus* Contaminants of Brewery Fermentations

F. B. STRANDSKOV and J. B. BOCKELMANN

The F. & M. Schaefer Brewing Co., Brooklyn, N. Y.

The growth of *Flavobacterium proteus* during a brewery fermentation is affected by the strain of brewer's yeast employed. The extent to which *F. proteus* can grow in a brewery fermentation appears to be related inversely to the rate at which the yeast strain employed can deaminate certain amino acids.

THE BREWING INDUSTRY commonly uses the yeast crop from a plant fermentation as inoculum in a subsequent fermentation. This process may be repeated several times before the yeast is replaced with a pure yeast culture and it usually results in a low level of bacterial contamination of the plant yeast.

The types of bacteria that grow sufficiently well during a brewery fermentation to establish a contamination are limited to those that will grow anaerobically, at a low pH level, and at the low temperature of the fermenting beer. It is also possible that the bacteria must be able to compete successfully with the yeast for certain nutrients that are utilized by the yeast during the fermentation (*7*) or that the bacterial growth is dependent on certain nutrients that are synthesized by the yeast and excreted into the fermenting beer (*3*). The bacteria that have been reported to contaminate brewer's yeast and brewery fermentations have been limited to gram-positive lactic acid rods and cocci, and a gram-negative rod *Flavobacterium proteus*.

Microscopic examination of brewer's yeast samples, from approximately 50 breweries, in the authors' laboratory during the past year has revealed that the bacterial contamination of a brewery yeast is usually limited to one morphological type. There are isolated cases where mixtures are found, but, in those cases, one type definitely predominates. That the contaminating bacteria found in any given yeast sample are not only of one morphological type, but also of a single physiological type is indicated by the data presented by Russell, Bhandari, and Walker (*4*). They found a tendency for bacterial strains isolated from any given brewer's yeast to have the same vitamin requirements for growth.

The variation in bacterial contaminants from one brewery to another and

the tendency of the bacteria that are found in any given brewer's yeast to be predominantly of one type indicate either that the yeast and fermenting beer in a brewery are exposed to only one type of bacteria that is capable of growing during the fermentation, or that there are fundamental differences in the fermentations, so that the fermenting beers are selective for one type of bacterial growth.

Because complete asepsis in a brewery is almost impossible, and because the authors' survey of the types of bacteria that are present in brewery yeast samples revealed that different bacterial types exist in yeast from breweries in the same general area, they feel that the most logical assumption is that the fermentations are exposed to a variety of bacterial types and that certain properties of the fermenting beers select the type of bacteria that will persist.

Such differences in brewery fermentations could be caused by variations in brewing procedures and formulas resulting in a variety of worts, by variations in the physical conditions of the fermentations, such as various pH and temperature levels, or by the effect of the nutritional requirements and general physiological reactions of the various strains of brewer's yeast. It is evident from the work of Sandegren and coworkers (*5*) and Barton-Wright (*2*), for instance, that the rate and extent to which certain amino acids are removed from fermenting beers varies with the yeast strain employed. Atkin and coworkers (*1*) have demonstrated that considerable differences exist in the vitamin requirements of brewer's yeast. The indications are, therefore, that the strain of brewer's yeast used in the brewery could influence the available nutrients and be a factor in determining the type of bacterial contaminant that can become established.

To study the effect of the brewer's yeast strain on the growth and survival of bacterial types during brewery fermentations, brewer's lager yeasts from three breweries were selected. These three strains of yeast were selected because they are used regularly in three breweries located within a radius of 20 miles and normally are contaminated with three distinctly different types of bacteria. The yeast strains are referred to as strains 1, 2, and 3.

Table I. Nutritional Requirements of *F. proteus* Strains 106 and 107

Strain Number	
106	*107*
Aspartic acid	Aspartic acid
Glutamic acid	Glutamic acid
Cysteine	Cysteine
Threonine	Threonine
Leucine	Leucine
Arginine	Arginine
Histidine	Histidine
Uracil	Uracil
Adenine	

The contaminant normally found in yeast 1 is a gram-negative rod *F. proteus;* that in yeast strain 2 is a gram-positive rod along with a few gram-negative rods of the same type as those found in yeast strain 1; and that of yeast strain 3 is a gram-positive rod which is different from the gram-positive rod found in yeast strain 2, plus a few gram-positive cocci. The observed differences in the two gram-positive rods are general morphology, and the rate and extent to which they will grow in a yeast extract, dextrose broth. The present report is concerned with the ability of the two strains of *F. proteus* isolated from yeast strains 1 and 2 to establish themselves in fermentations in which the three selected yeast strains are used.

The bacteria were isolated on a selective medium (*9*). The *F. proteus* strains isolated from yeast strains 1 and 2 are labeled 106 and 107, respectively. The data in Table I show that the amino acid requirements for growth of these two bacterial strains in a synthetic medium are similar. Strain 106 requires uracil and adenine, while strain 107 requires only uracil in addition to the amino acids.

The individual bacterial strains were added to pure cultures of the three selected brewer's yeast strains to give approximately five bacterial cells per 10,000 yeast cells. Fermentations using these artificially contaminated yeasts were carried out in 3-liter laboratory fermenters in which the authors have previously demonstrated that the fermentation proceeds in a normal manner (*8*). Daily bacterial counts on a selective medium (*9*) were made of the fermenting beers to determine the nature of the growth pattern of the two bacterial strains in the fermentations of each of the three yeast strains.

The data in Figures 1 and 2 show the rate at which the *F. proteus* strains 106 and 107 populations increase during successive fermentations in which each of the three yeasts are used. The data for the yeast strain 1 fermentations show a rapid increase in the number of bacteria during the first few fermentations until a maximum is reached, after which it remains constant. This is in agreement with previously published data (*6*). Although the maximum growth level of *F. proteus* strain 107 in yeast strain 1 fermentations is slightly lower than that obtained with *F. proteus* strain 106, the same general pattern does exist.

The growth of these two strains of *F. proteus* is considerably less in fermentations carried out with yeast strains 2 and 3, although they are able to establish growth and to multiply sufficiently during the fermentation to give a constant level of growth in successive fermentations. It is of interest to note that such a contamination can become established in yeast strain 3 fermentations, as this type of bacterial growth does not normally occur in this yeast.

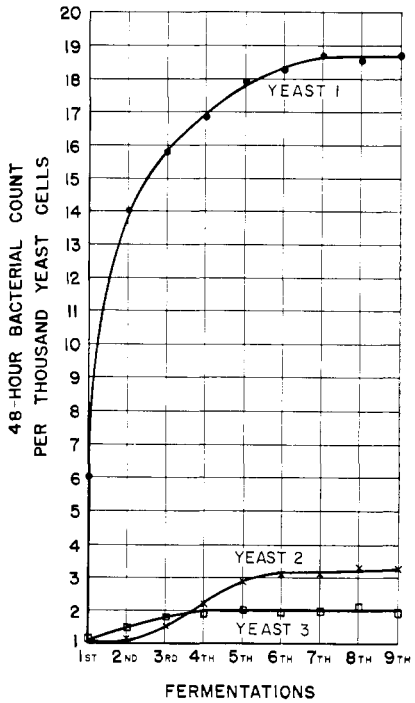

Figure 1. Increase in *F. proteus* 106 population during successive fermentations

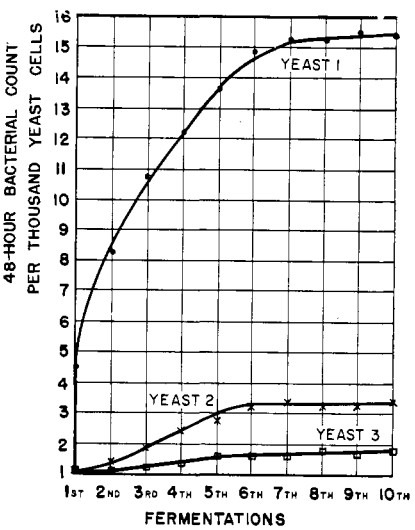

Figure 2. Increase in *F. proteus* 107 population during successive fermentations

It has been demonstrated that the rate of fermentation and the final pH of the fermented beer are affected by *F. proteus* strains (*8*). The data in Tables II and III show that *F. proteus* strains 106 and 107 have approximately the same affect on the fermentations. The degree to which the fermentation is affected is directly related to the level of the bacterial contaminant, as shown in Figures 1 and 2. The data are an average of

four successive experiments, during which the 48-hour bacterial growth level was at its maximum and constant.

In these experimental fermentations the physical and chemical properties of the wort, as well as the physical conditions of the fermentations, were constant. The authors, therefore, assume that some variation in the yeast metabolism which affects the fermenting beer is responsible for the difference in growth of *F. proteus* in the three fermentations.

Table II. Effect of *F. proteus* 106 Contamination on Fermentations of Yeasts 1, 2, and 3

	48-Hour pH		48-Hour Gravities, Degrees Plato	
Yeast	Pure yeast fermentation	Contaminated fermentation	Pure yeast fermentation	Contaminated fermentation
1	4.56	4.78	9.78	9.93
2	4.56	4.66	9.80	9.82
3	4.57	4.63	9.82	9.79

Table III. Effect of *F. proteus* 107 Contamination on Fermentations of Yeasts 1, 2, and 3

	48-Hour pH		48-Hour Gravities, Degrees Plato	
Yeast	Pure yeast fermentation	Contaminated fermentation	Pure yeast fermentation	Contaminated fermentation
1	4.56	4.76	9.78	9.91
2	4.56	4.68	9.80	9.84
3	4.57	4.61	9.82	9.80

Table IV. Vitamin Requirements for Yeast Growth in Synthetic Medium

Yeast	Vitamin Requirement
1	Pantothenic acid, inositol, and biotin
2	Pantothenic acid, inositol, and biotin
3	Pantothenic acid and biotin

Table V. Growth of Yeast Strains 1, 2, and 3 in a Medium Which Contains Only Ammonia as a Source of Nitrogen

	Yeast Solids, Mg./Ml., After	
Yeast	48 hours	72 hours
1	0.34	3.78
2	0.30	3.38
3	1.25	5.10

Table VI. Yeast Growth on Single Sources of Nitrogen

	Growth on Amino Acids / Growth on Ammonium Chloride × 100		
Amino Acid	Yeast 1	Yeast 2	Yeast 3
Alanine	15	10	33
Aspartic acid	6	33	97
Glutamic acid	13	33	48
Leucine	3	6	16
Isoleucine	5	10	15
Methionine	2	2	9
Arginine	27	10	14
Valine	2	29	11
Glycine	3	5	11
Threonine	5	1	4
Proline	6	5	19
Serine	29	27	84

The vitamin requirements for growth of the three yeast strains were determined according to the method of Atkin and coworkers (*1*). The data in Table IV show the vitamin growth requirements of the three yeast strains when they are grown on hydrolyzed casein as a source of nitrogen and glucose as a carbon source. Strains 1 and 2 require pantothenic acid, inositol, and biotin for growth, while strain 3 requires only pantothenic acid and biotin. The same results are obtained when ammonia is substituted for hydrolyzed casein as a source of nitrogen.

Ammonia can be utilized by brewer's yeast as the sole source of nitrogen for the synthesis of all the complex compounds required for cell structure and cell metabolism. A number of amino acids can also be used for this purpose (*7, 10*). When single amino acids are supplied, the rate of growth is less rapid than it is when ammonia is present. The apparent reason is that the amino acids must first be deaminated to make ammonia available for the required synthesis and that the rate of growth is, therefore, limited by the rate of deamination.

The data in Table V show the relative rates of growth of yeast strains 1, 2, and 3 in a medium in which ammonia is supplied as a source of nitrogen. The growth of strain 3 is considerably more rapid than the growth of strains 1 and 2.

In Table VI data are presented showing the rate of growth of these three yeast strains on single amino acids as sources of nitrogen, and as percentages of the rate of growth of the yeast on ammonia as the sole source of nitrogen. In each case the concentration of amino acids was adjusted so that the concentration of amino nitrogen was equal to the concentration of ammonia nitrogen in the controls. The rate of growth on an amino acid as the sole source of nitrogen is less than the rate of growth of the three yeast strains on ammonia.

If the growth rates obtained on single amino acids are limited by the rates at which these amino acids are deaminated, then it appears that the deaminating enzymes of yeast 3 are much more active than those of yeast strains 1 and 2, and that those of yeast strain 2 are, in general, equal to or greater in activity than those of strain 1. One exception is the enzyme responsible for the deamination of arginine. This enzyme is most active in yeast strain 1. None of the yeasts could attack lysine, histidine, phenylalanine, tyrosine, tryptophan, cysteine, or hydroxyproline.

Discussion

The rate and extent of growth of *F. proteus* in experimental brewery fermentations is influenced by the strain of brewer's yeast employed. The data presented show an inverse relationship between the rate of growth of the individual yeast strains on some amino acids in media which contain single amino acids as the sole source of nitrogen, and the extent to which *F. proteus* can grow in fermentations in which these yeasts are used. Two of the amino acids that support more rapid growth of yeast strains 2 and 3, than of yeast strain 1, are glutamic acid and aspartic acid. Although other amino acids are also deaminated more rapidly by yeast strains 2 and 3 than by yeast strain 1, the activity of the yeasts on these two amino acids is of particular interest, as it has been shown that *F. proteus* synthesizes these two amino acids very slowly (*7*). Assuming that the deamination of these amino acids by yeast strains 2 and 3 during a fermentation is also more rapid than it is in a yeast strain 1 fermentation, then this could be the factor responsible for the limited amount of *F. proteus* growth obtained in a fermentation in which these two yeasts are used, as compared with the growth of *F. proteus* in a fermentation carried out with yeast strain 1.

Acknowledgment

The authors acknowledge the technical assistance of J. A. Brescia, John Dwyer, R. E. Miranda, William Tirado, and R. C. Turczany.

Literature Cited

(1) Atkin, L., Gray, P. P., Moses, W., Feinstein, M., *Wallerstein Labs. Communs.* **12,** No. 37, 153 (1949).
(2) Barton-Wright, E. C., *Ibid.*, **15,** No. 49, 115 (1952).
(3) Challinon, S. W., Rose, A. H., *Nature* **147,** 877 (1954).
(4) Russell, C., Bhandari, R. R., Walker, T. K., *J. Gen. Microbiol.* **10,** 371 (1954).
(5) Sandegren, E., Enebo, L., Guthenberg, H., Ljungdahl, L., *Am. Soc. Brewing Chemists Proc.* **1954,** 63.
(6) Strandskov, F. B., Baker, H. W., and Bockelmann, J. B., *Wallerstein Labs. Communs.* **16,** No. 54, 261 (1953).
(7) Strandskov, F. B., Bockelmann, J. B., *Am. Soc. Brewing Chemists Proc.* **1955,** 36.
(8) Strandskov, F. B., Bockelmann, J. B., J. AGR. FOOD CHEM. **1,** 1219 (1953).
(9) Strandskov, F. B., Bockelmann, J. B., *J. Inst. Brewing* **57,** No. 2, 123 (1951).
(10) Thorne, R. S. W., *J. Inst. Brewing* **55,** No. 4, 201 (1949).

Received for review February 10, 1956. Accepted July 18, 1956.