

Yeasts from Commercial Meat Brines¹

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Little information is available on the nature of the yeast flora associated with meat brines; particularly brines from commercial sources. Sturges (1923) isolated yeasts from ham brines and referred to them as "torula". Mrak and Bonar (1939) investigated 28 cultures isolated from 27 samples of various brined and pickled products; two cultures of *Debaryomyces* were obtained from the single sample of ham brine examined. Graham and Hastings (1942) isolated six cultures of film-forming yeasts from the surface of rennet brines, all of which were placed in two species of *Debaryomyces*. Unpublished work in this Department on experimentally brined bacon sides has shown that progressive increases in yeast populations occur in the brine after the first 2 weeks of cure, and maximum populations of 6 to 7 million yeasts per ml were reached by the end of a prolonged curing period. The predominating yeast species found in subsurface samples was a nonfilm-forming

species of *Debaryomyces* which was classified as *Debaryomyces klockeri*.

The purpose of the present investigation was to obtain additional information on the nature of the yeast flora associated with brined meat cured under commercial conditions.

MATERIALS AND METHODS

Brine samples from nine casks of brined meat were obtained from a commercial meat packing plant located in Richmond, Virginia. The brines were streaked in duplicate onto previously poured plates of acidified dextrose agar (Etchells and Jones, 1946) containing 6 to 8 per cent salt by weight. Also, samples from each cask were placed in sterile 150-ml flasks and refrigerated (35 F). Heavy, wrinkled films developed on all samples and these films were streaked on the above mentioned medium. Well-isolated colonies from the brines and films were restreaked for purification and transferred to vegetable-juice agar slants. This yeast sporulation medium was prepared according to Wickerham, Flickinger, and Burton (1946) except for minor changes suggested by Etchells and Bell (1950a). A total of 89 yeast isolates were obtained during the investigation and information as to their source is presented in table 1.

The methods and classification systems employed

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for the taxonomic placement of the isolates were essentially those outlined by Stelling-Dekker (1931). Certain modifications of these methods were used; these have been described by Etchells and Bell (1950 a, b) and Etchells, Costilow and Bell (1952), in connection with their taxonomic studies on subsurface and film-forming yeasts associated with the fermentation of commercially brined cucumbers. The carbon assimilation tests run and interpreted by Dr. L. J. Wickerham (USDA), Peoria, Illinois, were of much value in the identification of the nonfilm-forming yeast isolates.

RESULTS AND DISCUSSION

All of the 89 yeast isolates obtained in this study were found to belong to the genus *Debaryomyces*; 86 were placed as *D. membranaefaciens* var. *Hollandicus* Lodder⁴, and the remaining three were classified as *D. klockeri* Guill. et Péju. Information on the distribution of the isolates of each species, with respect to their occurrence in surface or subsurface samples, is given in table 2.

All 70 of the cultures isolated from films were *D. membranaefaciens* var. *Hollandicus*; also, 17 of the 20 cultures obtained from subsurface brine samples belonged to this species. Pictures of the heavy, wrinkled film formed by this yeast (and several other film-forming species) on brines, together with illustrations of colonial and cellular morphology have recently been published (Etchells, Bell, and Jones, 1953). The presence of this yeast in subsurface cucumber brine samples is usually associated with the heavy surface growth that has fallen or in some manner has been disturbed (Etchells, Costilow, and Bell, 1952). The same condition may have been present in the case of the subsurface meat brines, because the evidence at sampling indicated that the original films had been disturbed.

The isolation of only three cultures of *D. klockeri* from subsurface samples of meat brines may be attributed to the fact that only two of the nine brines sampled were over 10 days old. In the bacon studies referred to earlier, this species reached maximum populations of several millions per ml of brine after a prolonged curing period (112 days). However, only very low counts (200 to 1,000 per ml) were observed for the first 2 weeks. *D. klockeri* is a nonfermentative, nonfilm-forming species. It does, however, assimilate a large number of carbon compounds and is probably more widespread in meat

⁴ Since this work was completed, the Dutch workers (Lodder and Kreger-Van Rij, 1952) have proposed an emended genus of *Debaryomyces*. The variety of the species *D. membranaefaciens* has been included in the species *D. nicotianae*. However, our isolates do not conform with the description of this species because they assimilate lactose. If the new system were to be used, the isolates would probably be closer to *D. hansenii* than to *D. nicotianae*. For the present study we prefer to use the earlier classification of Stelling-Dekker (1931) and retain the name *D. membranaefaciens* var. *Hollandicus*.

TABLE 1. Origin of 89 yeast isolates from commercial meat brines

Cask No.	Type of Meat Brine Sampled	Age of Brine at Sampling	Brine pH	Salt Concentration by Weight	No. of Isolates Obtained From:	
					Sub-surface brine samples	Films on brines
		days		per cent		
1	Ham	3	5.70	9.8	2	6
2	Ham	3	5.95	8.9	2	9
3	Ham	8	5.95	10.1	1	9
4	Used ham brine	—	5.55	8.9	3	8
5	Beef tongue	3	6.10	8.6	0	8
6	Beef tongue	15	5.90	8.7	3	6
7	Bacon sides	5	6.12	10.9	3	9
8	Bacon sides	5	6.05	10.8	2	9
9	Canadian bacon	17	5.78	8.6	3	6
Subtotal.....					19	70
Total.....					89	

TABLE 2. Classification and distribution of yeast isolates from commercial meat brines

Classification	Number of Isolates Obtained From:		Total Number
	Subsurface brine samples	Films on brines	
<i>D. membranaefaciens</i> var. <i>Hollandicus</i>	16	70	86
<i>D. klockeri</i>	3	0	3
Total.....	19	70	89

brines and refrigerated meat products than the present study indicates.

Judging from the literature (Hof, 1935; Mrak and Bonar, 1939; Graham and Hastings, 1942; Etchells and Bell, 1950b; Zenitani, 1952) and the current report, it seems apparent that species of *Debaryomyces* are the most widely distributed yeasts associated with food brines. Furthermore, one species (*D. membranaefaciens* var. *Hollandicus*) appears to be responsible for most film formation on such brines. The *Debaryomyces* exhibit high tolerance to salt and organic acids; these characteristics, coupled with their ability to grow well at low temperature (35 F) and assimilate a variety of carbon compounds, are important factors responsible for the prevalence of these yeasts in connection with foods that are preserved by salting and brining.

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SUMMARY

A total of 89 yeast isolates, all belonging to the genus *Debaryomyces* were obtained from nine casks of commercially brined meat (hams, beef tongues, bacon sides and Canadian bacon). Seventy of the isolates came from surface films on the brines and 19 were from subsurface brine samples. Eighty-six of the cultures were identified as *Debaryomyces membranaceus* var. *Hollandicus* Lodder and were responsible for film formation on the brines. The remaining three cultures were placed as *Debaryomyces klockeri* Guill. et Péju. These isolates were nonfilm-forming and were isolated from subsurface brine samples.

REFERENCES

- ETCHELLS, J. L., AND BELL, T. A. 1950a Classification of yeasts from the fermentation of commercially brined cucumbers. *Farlowia*, **4**, 87-112.
- ETCHELLS, J. L., AND BELL, T. A. 1950b Film yeasts on commercial cucumber brines. *Food Technol.*, **4**, 77-83.
- ETCHELLS, J. L., BELL, T. A., AND JONES, I. D. 1953 Morphology and pigmentation of certain yeasts from brines and the cucumber plant. *Farlowia*, **4**, 265-304.
- ETCHELLS, J. L., COSTILOW, R. N., AND BELL, T. A. 1952 Identification of yeasts from commercial cucumber fermentations in northern brining areas. *Farlowia*, **4**, 249-264.
- ETCHELLS, J. L., AND JONES, I. D. 1946 Procedure for bacteriological examination of brined, salted, and pickled vegetables and vegetable products. *Am. J. of Public Health*, **36**, 1112-1123.
- GRAHAM, V. E., AND HASTINGS, E. G. 1942 Studies on film-forming yeasts. II. Film-forming yeast in rennet brine. *Can. J. Research, C*, **20**, 63-67.
- HOF, T. 1935 Investigations concerning bacterial life in strong brines. *Rec. trav. botan. néerl.*, **32**, 92-173.
- LODDER, J., AND KREGER-VAN RIJ, N. J. W. 1952 *The Yeasts—A Taxonomic Study*. North-Holland Publishing Co., Amsterdam.
- MRAK, E. M., AND BONAR, L. 1939 Film yeasts from pickle brines. *Zentr. Bakt. Parasitenk.*, **II**, **100**, 289-294.
- STELLING-DEKKER, N. M. 1931 *Die Hefesammlung des "Centraalbureau voor Schimmelcultures."* I Teil. *Die sporogenen Hefen*. Amsterdam.
- STURGES, W. S. 1923 Studies on halophilic micro-organisms. The flora of meat curing solutions. *Abst. Bacteriol.*, **7**, 11.
- WICKERHAM, L. J., FLICKINGER, M. H., AND BURTON, K. A. 1946 A modification of Henrici's vegetable-juice sporulation medium for yeasts. *J. Bacteriol.*, **52**, 611-612.
- ZENITANI, B. 1952 Yeasts occurring in fishery-fermentation products. Part I. On the generic classification of true yeast in "Shiokara". *Science Bull. Fac. of Agr., Kyushu Univ.*, **12**, 247-253.