4(1):249–264 FARLOWIA OCTOBER, 1952

IDENTIFICATION OF YEASTS FROM COMMERCIAL CUCUMBER FERMENTATIONS IN NORTHERN BRINING AREAS[1]

J. L. ETCHELLS, R. N. COSTILOW, AND T. A. BELL

Bureau of Agricultural and Industrial Chemistry,
U. S. Department of Agriculture and
North Carolina Agricultural Experiment Station
Raleigh, North Carolina

Cucumbers for pickling represent an important agricultural commodity — ranking fourth in acreage and sixth in value of all horticultural crops for processing. The annual crop of pickling cucumbers is about 10 million bushels; of this amount, approximately 8 to 8.5 million bushels are brine-cured and the balance goes to fresh-pack or pasteurized products. About one-fourth of the crop is brined in the Southeastern States; and Indiana, Michigan, and Wisconsin brine approximately one-half.

For the past several years this Bureau, in cooperation with the Department of Horticulture of the North Carolina Agricultural Experiment Station, has been investigating the microbiological changes occurring during the fermentation of cucumbers brined under southern conditions. More recently part of this cooperative work has centered around that portion of the fermentation caused by yeast activity. Particular emphasis has been placed on the identity of the individual species comprising the total yeast population. We are interested in obtaining basic information of this type for the major cucumber pickling areas of the country in an effort to improve existing brining procedures and minimize losses caused by (1) enzymatic softening of salt-stock; and (2) the formation of bloaters (hollow salt-stock). Most yeasts of cucumber brine origin possess two important qualities that would be required of any microbial group associated with the two important types of economic loss mentioned above. These qualities are high tolerance to salt and high tolerance to acid.

In a recent report (Etchells and Bell, 1950 *a*), 1,444 yeast isolates associated with the gaseous fermentation of cucumbers under conditions typical of southern brining areas (e.g. North Carolina) were classified. During the period when isolations were made, the brine strength of the fermentations was within the range of 10 to 18 percent by weight. It was found that the isolates could be reduced to the following six genera in the order of frequency of isolation: *Torulopsis*, 721 isolates; *Brettanomyces*, 588; *Zygosaccharomyces*, 59; *Hansenula*, 49; *Torulaspora*, 6; *Kloeckera*,

[1] This study was carried out under a cooperative project with the Department of Horticulture of the North Carolina Agricultural Experiment Station. Published with the approval of the Director, North Carolina Agricultural Experiment Station as Paper No. 383 of the Journal Series.

1; and 20 cultures not fully classified. Bloater formation in the vats of salt-stock cucumbers examined was attributed to the gaseous yeast fermentation. The proportion of bloaters found in seven vats of large-sized cucumbers ranged from 23 to 54 percent.

Although the above study definitely established that certain yeast species were related to bloater formation under southern fermentation conditions, no incrimination of these species has been demonstrated to date (Etchells and Bell, 1951) in connection with the type of enzymatic softening of cucumber salt-stock described by Bell, Etchells, and Jones (1950). However, several yeast species (all from sources other than cucumber brines) have been reported (Luh and Phaff, 1948; Hall and Teunisson, 1948; Etchells and Bell, 1951) as being capable of producing a pectin-splitting enzyme similar to the one responsible for softening of salt-stock (Bell, Etchells, and Jones). Such yeasts, growing in commercial cucumber brines, could contribute materially to the softening type of spoilage. Continued isolation and identification of yeasts from cucumber fermentations in the major brining areas of the country may reveal species capable of glycosidic hydrolysis of pectin.

Previous studies on the yeasts responsible for the gaseous fermentation of cucumbers have been limited to conditions typical of southern states. The present investigation was undertaken (1) to establish whether yeast activity represents a part of the cucumber fermentation under conditions typical of northern brining areas represented by the states of Indiana, Michigan and Wisconsin, and (2) if so, to determine the nature and sequence of the principal yeast species present.

EXPERIMENTAL PROCEDURE

During a 3-year period (1948–50) 155 brine samples were collected from 21 commercial cucumber brining stations, operated by eight pickle companies in Indiana, Michigan, and Wisconsin. The location of brining stations visited in each state, and the number of brining seasons each station was visited, are given in table 1. During the 1948 and 1949 seasons, the brines were collected during the first week in November and represented the middle to late periods of fermentation; during the 1950 season, the samples were taken during September, 4th to 9th, and covered the early period of fermentation. The brine temperatures of the fermentations sampled during the 1950 season were as follows: Indiana brines, 19 to 20°C; Michigan and Wisconsin brines, 15.5 to 20°C. Brine temperatures were not taken during the 1948 and 1949 sampling periods. A total of 452 yeast isolates were obtained during the investigation and information as to their source is presented in table 2. A general picture with respect to total yeast populations, rate of increase in brine strength, and chemical changes with respect to brine acidity and pH, is presented in figure 1. The curves represent mean values for all brines collected during the 3-year period.

TABLE 1. LOCATION OF NORTHERN CUCUMBER BRINING STATIONS FROM WHICH BRINE SAMPLES WERE COLLECTED DURING 1948, 1949, AND 1950.

State	Brining Station	General Location of Station in Each State	Brining Seasons During Which Station Was Visited	
	code	*no.*	*no.*	*yrs.*
Indiana	NJ	North West	3	1948–50
	KR	North West	3	1948–50
	KO	North West	1	1948
State Total		3		
Michigan	LT	South Central	3	1948–50
	LA	South Central	3	1948–50
	CL	Central	2	1949–50
	BA	South West	1	1948
	RD	South Central	1	1948
	SG	East Central	1	1950
	NL	Central	1	1950
	SN	East Central	1	1950
	CS	West Central	1	1950
	LV	West Central	1	1950
	CC	East Central	1	1950
State Total		11		
Wisconsin	BR	Central	2	1948; 50
	WY	Central	2	1948; 50
	PL	South East	2	1948; 50
	WA	Central	2	1948; 50
	OX	Central	1	1948
	GB	East Central	1	1950
	WU	Central	1	1950
	NK	Central	1	1950
State Total		8		
Total		22		

Of the 155 individual vats sampled, about one-third were under outside conditions and the remainder were sheltered from the weather and direct sunlight. Vats so protected afford excellent conditions for good growth of yeast species responsible for film formation on the brine surface (Etchells and Bell, 1950 *b*). More important is the fact that contamination of subsurface brine samples with species of film yeasts can become a problem unless care is taken with the sampling procedure (Etchells and Jones, 1946; Etchells and Bell, 1950 *a*). In the present study, less than 3 percent of the 452 isolates were classified as film yeasts which originated from surface growth on sheltered vat brines. The 12 film yeast isolates (7 *Candida* and 5 *Debaryomyces*) came from seven brines; five were collected during the 1949 season; and two from 1950.

The brine samples were collected, plated, and counted according to methods previously described by Etchells (1941) and revised by Etchells and Jones (1946), and Etchells and Bell (1950 *a*). All brines were plated on acidified glucose agar containing 6 to 8 percent salt by weight and the plates from the high dilutions set aside for picking representative colonies. These colonies were identified and the proportion of each species

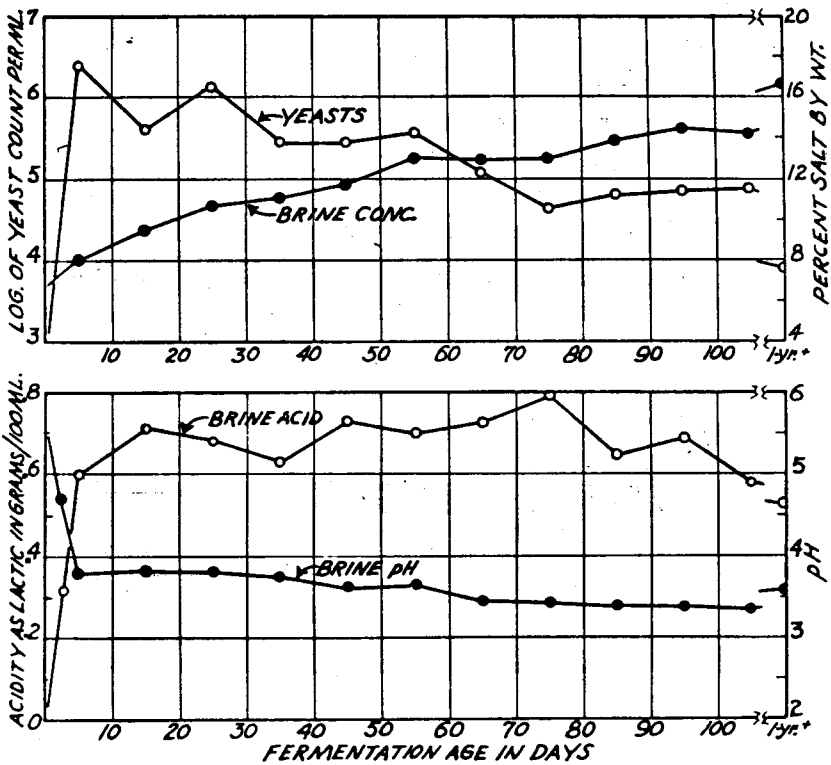

Figure 1. Total yeast populations, increase in brine concentration, brine acidity and pH of northern cucumber fermentations.

present was assigned a value which gave an estimate of their numbers in the brine sample. The methods and classification systems employed for the 452 isolates were essentially those outlined by the Dutch workers; Stelling-Dekker (1931), Lodder (1934), Diddens and Lodder (1942), and Custers (1940). Bedford's (1942) classification system was used for the genus *Hansenula*. For confirming the ability of isolates of certain genera (e.g. *Hansenula* and *Brettanomyces*) to utilize nitrate, the assimilation test of Wickerham (1946, 1951) was particularly useful. Certain other modifications and additions were made in connection with the taxonomic tests employed by the Dutch group. These have been fully described by Etchells and Bell (1950 *a*, *b*).

TABLE 2. ORIGIN OF 452 YEAST ISOLATES FROM 155 CUCUMBER BRINES OBTAINED FROM 22 BRINING STATIONS LOCATED IN INDIANA, MICHIGAN, AND WISCONSIN (1948–50 BRINING SEASONS).

State and Brining Season	Brining Stations Visited [1]	Brine Samples Obtained	Chemical Examination of Brines: Salt Conc. by Wt.	Chemical Examination of Brines: Acidity as Lactic	Chemical Examination of Brines: pH	Fermentation Period Covered by Samplings [2]	Yeast Isolates Obtained
	number	*number*	*percent*	*percent*		*days*	*number*
Indiana							
1948	3	11	13–17	0.46–.88	4.1–3.4	54–97	31
1949	2	21	13–15	.34–.78	3.6–3.2	66–105	56
1950	2	16	4–12	.27–.85	3.9–3.7	4–55	59
	7	48					146
Michigan							
1948	4	20	11–18	0.38–.88	4.2–3.1	39–94	57
1949	3	16	14–17	.52–.83	3.7–3.3	70–98	33
1950	9	32	7–12	.31–.92	4.8–3.6	2–44	97
	16	68					187
Wisconsin							
1948	5	21	11–14	0.62–1.16	3.6–3.2	48–100	58
1949	0	0	....	....	....	....	0
1950	7	18	8–13	.41–.98	4.1–3.6	13–38	61
	12	39					119
Overall	35	155	4–18	0.27–1.16	4.8–3.1	2–105	452

[1] Refers to total number visits to 22 individual brining stations operated by 8 pickle plants and located as follows: Indiana, 3 stations; Michigan, 11; and Wisconsin, 8.

[2] Brine samples were obtained from several vats in Indiana and Michigan during 1948 and 1949 after 12–14 months' storage.

RESULTS

The 452 isolates obtained during the 3-year study were reduced to eight generic groups as follows: I. *Brettanomyces*, 132 isolates (29.2%); II. *Torulopsis*, 103 (22.8%); III. *Torulaspora*, 68 (15.0%); IV. *Hansenula*, 59 (13.1%); V. *Saccharomyces*, s.g. *Zygosaccharomyces*, 46 (10.2%); VI. *Saccharomyces*, s.g. *Saccharomyces* s.s., 22 (4.9%); VII. *Candida*, 7 (1.5%); VIII. *Debaryomyces*, 5 (1.1%); and an unclassified group of 10 isolates. Species separation under each generic group is summarized in table 3. Information is also presented on species distribution for each brining season with respect to: (1) Number of isolates found; (2) number of vat brines where found; and (3) number of brining stations where found.

Predominating Genera

Group I, Genus *Brettanomyces* Kuff. et van Laer: Of the 132 isolates placed in this genus, 103 were identified as *B. versatilis* Etchells et Bell. This yeast was the most frequently isolated species obtained during the study and was widely distributed in the brines collected each season from all three states. *B. versatilis* was also found in several instances where the brine samples came from vats of salt-stock that were 12–14 months old. Twenty-nine isolates were closely related to *B. sphaericus* Etchells et Bell. Until further study can be made of this group, we prefer to list them as an unnamed variety of *B. sphaericus*. Most of these cultures were obtained in Indiana and Michigan during the 1949 and 1950 brining seasons.

Group II, Genus *Torulopsis* Berlese: The 103 cultures placed in this genus consisted of two species: *T. holmii* (Jörgensen) Lodder accounted for 67 cultures which, with the exception of one isolate obtained in 1948, came from brines collected from nine brining stations during the 1950 season. This species occurred chiefly in brines during the early part of the fermentation. The 36 isolates placed as *T. caroliniana* Etchells et Bell were obtained during two brining seasons (1948 and 1950), and came mostly from brines collected at two Indiana brining stations; however, a few cultures were isolated from Michigan brines.

Group III, *Torulaspora* Lindner: The 68 isolates placed in this genus were identified as *T. rosei* Guillermond and were obtained in about equal numbers from brines collected during the 1948 and 1950 seasons. This yeast was not found in the 1949 brines collected in the regular manner. The cultures were identical as far as cultural, morphological, fermentation, and carbon assimilation reactions were concerned, but differed with respect to sporulation behavior. Some sporulated readily; others produced typical copulation tubes but spores did not develop; still others produced neither copulation tubes nor spores.[2]

Group IV, Genus *Hansenula* Sydow: This genus was represented by

[2] Dr. L. J. Wickerham kindly checked representative cultures of our *T. rosei* collection and found the carbon assimilation patterns to be the same.

TABLE 3. CLASSIFICATION AND DISTRIBUTION OF 452 YEAST ISOLATES FROM 155 NORTHERN CUCUMBER BRINES.

Generic Group (Number and Percent of Isolates), and Species	Classification of Isolates		Distribution of Isolates According to: Brining Season			Vat Brines			Brining Stations		
			1948	1949	1950	1948 (52) [1]	1949 (37) [1]	1950 (66) [1]	1948 (12) [2]	1949 (5) [2]	1950 (18) [2]
	no.	*percent*	*no. isol.*	*no. isol.*	*no. isol.*	*no. vats*	*no. vats*	*no. vats*	*no. sta.*	*no. sta.*	*no. sta.*
I. BRETTANOMYCES (132; 29.2%)											
B. versatilis	103	22.8	43	27	33	21	17	19	12	5	8
B. sphaericus var.	29	6.4	0	15	14	0	12	9	0	4	5
II. TORULOPSIS (103; 22.8%)											
T. holmii	67	14.8	1	0	66	1	0	23	1	0	9
T. caroliniana	36	8.0	16	0	20	6	0	7	2	0	3
III. TORULASPORA (68; 15.0%)											
T. rosei	68	15.0	35	0	33	17	0	14	7	0	7
IV. HANSENULA (59; 13.1%)											
H. subpelliculosa	59	13.1	19	24	16	16	15	9	9	4	7
V. ZYGOSACCHAROMYCES s.g. (46; 10.2%)											
Z. halomembranis	28	6.2	7	14	7	4	8	7	2	2	2
Z. globiformis	14	3.1	11	0	3	7	0	2	5	0	1
Z. sp. A	3	0.7	0	1	2	0	1	2	0	1	1
Z. pastori	1	0.2	0	1	0	0	1	0	0	1	0
VI. SACCHAROMYCES s.g. (22; 4.9%)											
S. globosus	22	4.9	13	0	9	6	0	4	4	0	3
VII. CANDIDA (7; 1.5%)											
C. krusei	7	1.5	0	0	7	0	0	2	0	0	2
VIII. DEBARYOMYCES (5; 1.1%)											
D. membran. var. *Holl.*	5	1.1	0	5	0	0	5	0	0	2	0
UNCLASSIFIED	10	2.2	1	2	7	1	1	6	1	1	6
	452	100	146	89	217						

[1] Number of vat brines collected each year; values listed in each column represent the number of brines where each species was found.

[2] Number of individual brining stations visited each year; values listed in each column represent the number of brining stations where each species was found.

59 cultures of *H. subpelliculosa* Bedford, obtained from representative brining stations in Indiana, Michigan, and Wisconsin. This species was consistently found during all three brining seasons. The cultures were divided into two groups; those that fermented maltose, and those that gave a weak to negative fermentation test for this sugar. Etchells and Bell (1950 *a*) considered that the fermentation of maltose was a weak characteristic for this species because of the variable reactions obtained when a sufficient number of cultures were studied. More recently, Wickerham (1951) also found that *H. subpelliculosa* gave a variable maltose fermentation.

Group V, Genus *Saccharomyces* (Meyen) Reess; subgenus *Zygosaccharomyces* Barker; The 46 isolates placed in this subgenus were allocated to the following four species: *Z. halomembranis* Etchells et Bell, 28 cultures; *Z. globiformis* Kr. et Kb., 14; *Z.* species A, 3; and *Z. pastori* Guillermond, 1. The species *Z. halomembranis* was consistently found in the brines of two Indiana stations during the 3-year study. The isolates placed as *Z. globiformis* were similar but not identical to this species. However, the characteristics were essentially the same as those described in detail by Etchells and Bell (1950 *a*) for their *Z.* species A, obtained from the fermentation of cucumbers brined under southern conditions.

Group VI, Genus *Saccharomyces* (Meyen) Reess; subgenus *Saccharomyces* s.s.: This subgenus was represented by 22 cultures of *S. globosus* Osterw. obtained during the 1948 and 1950 seasons from a total of seven brining stations, two in Wisconsin and five in Michigan. This particular yeast constitutes the chief difference with respect to the individual species found in northern brines as compared to those found in southern brines (Etchells and Bell, 1950 *a*). Although *S. globosus* was not found as frequently as certain of the other species, four active gaseous fermentations were attributed to this yeast. They were observed in 1948 at two brining stations in Michigan and one in Wisconsin, in brines that were 43, 44, 99, and 100 days old.

Group VII, Genus *Candida* Berkhout emend. Diddens et Lodder: The seven cultures placed in this genus were identified as *C. krusei* (A. Cast.) Berkhout and came from two 1950 brines of low salt content (4 and 8 percent). The presence of these isolates in the subsurface brine samples is attributed to film formation on the two sheltered vat brines from which they were obtained. This species was found by Etchells and Bell (1950 *b*) to be associated with film formation on commercial pickle brines of about 5 percent strength.

Group VIII, Genus *Debaryomyces* Klöcker: The five isolates of this genus were identified as the film yeast *D. membranaefaciens* var. *Hollandicus* Lodder. The cultures were obtained from five brines collected during the 1949 season at two brining plants in Indiana. The vats at both stations were sheltered from direct sunlight and provided excellent conditions for film yeast development on the surface of the brines. It is our opinion

that the five *Debaryomyces* cultures originated from the surface growth on the brines.

Unclassified Group: Ten of the 452 cultures obtained during the study have not been fully classified. Seven of the unidentified isolates came from six brines of the 1950 season; two were obtained during 1949; and one in 1948.

Populations of Individual Species

The results for the 3-year study with respect to estimated population trends for the different yeast species occurring in the 155 brines are shown in figure 2. In the preparation of these scatter diagrams, the individual values plotted represent the estimated yeast count as to species in the various brines. The general trend for species activity in each case is represented by the curve drawn through the individual values, calculated from means obtained at 10-day intervals throughout the fermentation period.

Two yeasts, *T. holmii* and *B. versatilis*, show rather clear-cut fermentation trends. The first species occurred chiefly during the early portion of the fermentation; the second dominated the late portion. As mentioned earlier, *T. holmii*, with one exception, was not found in the brines collected during the 1948 and 1949 brining seasons. A possible reason for this was that these brines represented the middle to late periods of fermentation (40 days and beyond). Thus, on the basis of the 1950 season's results, *T. holmii* would not be expected to be found in any large numbers in brines that were over 30 to 40 days in age.

The consistent occurrence of *Hansenula subpelliculosa* from brining stations representative of the three states throughout the three pickling seasons is sufficient to include this species as one of the principal species contributing to the yeast fermentation. Perhaps the same is true for *Torulaspora rosei*, even though it was not obtained from brines collected during the 1949 season. However, during 1949, brines were not obtained from Wisconsin (Cf. tables 1 and 2); whereas the four stations sampled in that state during 1948 accounted for almost three quarters of the *T. rosei* isolates obtained from all 12 stations sampled. Further, the fermentation trend (Cf. figure 2) for this yeast is not fully characterized because plate counts were not made on 11 of the 1948 Wisconsin brines where *T. rosei* represented almost 60 percent of the isolations.

The activity of *Zygosaccharomyces halomembranis* in northern brines is based chiefly on consistent isolations of this species from two Indiana stations during the three brining seasons. Etchells and Bell (1950 *b*) identified this yeast as one of the species responsible for films on sheltered commercial vat brines located in Indiana, Michigan and Wisconsin. This species ferments glucose and maltose strongly and it is our opinion that it exists in commercial brines both as an oxidative and fermentative species, thus accounting for its occurrence in subsurface fermenting brines as

well as for its occurrence on the surface of sheltered vat brines. Laboratory tests confirm this assumption.

The dominant position occupied by *Torulopsis caroliniana* in the early portion of southern cucumber fermentations (Etchells and Bell, 1950 *a*) is in sharp contrast to the relatively minor role exhibited by this species

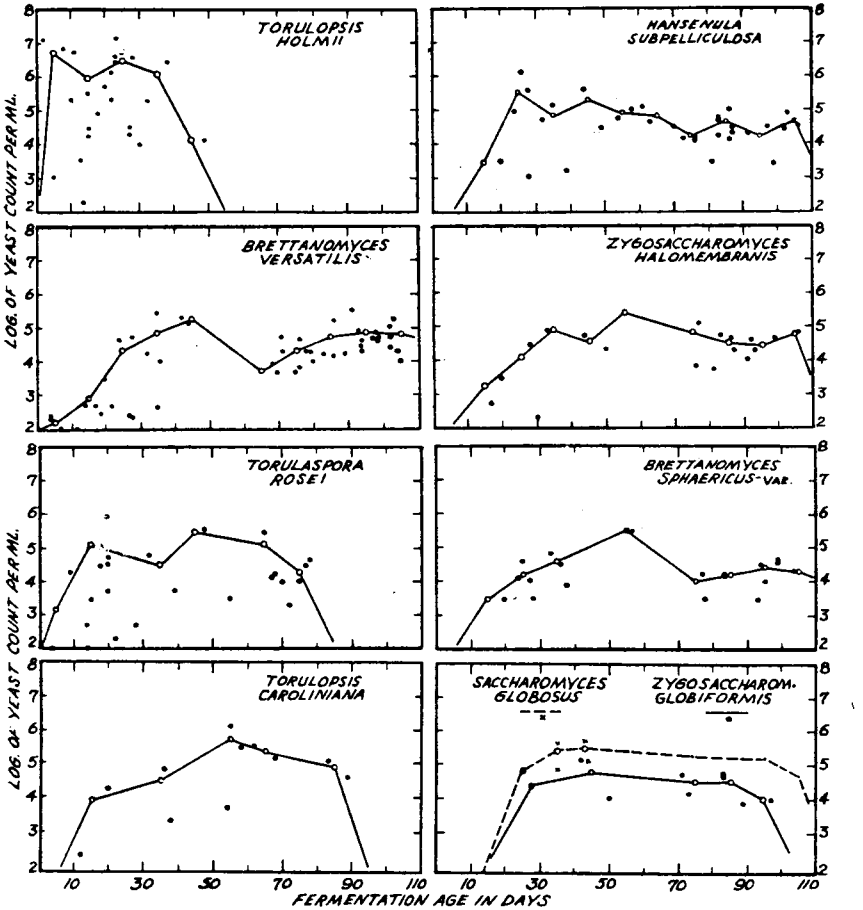

Figure 2. Population trends for nine species and one variety of six yeast genera occurring in cucumber fermentations brined under northern conditions.

in the current study on northern brines. It appears that *T. holmii* is the major species contributing to the early part of the northern fermentations, and *T. caroliniana* is of minor importance. *T. caroliniana* was found for a much longer period in northern than in southern brines; about 90 days versus 40 days. This is attributed, in part, to the fact that even though the former brines generally contain more total acid than the latter, they

also are more highly buffered and thus are not as toxic for *T. caroliniana* which is less acid tolerant than the other brine yeast species. Further, there is reason to believe that in some northern brines, *T. caroliniana* may have been missed because they grew out very poorly on the acidified glucose agar containing salt. Colonies which were about 0.1–0.2 mm. in diameter were first considered to be poorly developed lactobacilli colonies growing out, but later proved to be *T. caroliniana*. Upon transfer to less acid media, these cultures gave growth typical of the species. However, since all brines were first screened for the numbers and morphological types of live and dead yeast cells by the direct microscopic counting technique, it is doubtful if many isolates representing substantial populations of this species in brines were overlooked. *T. caroliniana*, because of its extremely small size, can usually be recognized in brine fermentations by microscopic examination.

Of the remaining three species (shown in figure 2), two, the unnamed variety of *Brettanomyces sphaericus* and *Saccharomyces globosus*, should be discussed briefly. The former species occurred in the brines during essentially the same period as did *B. versatilis* although not in as large numbers, or as consistently on a seasonal basis. Further, *B. sphaericus* var. was the only species, other than *B. versatilis*, that was found in brines after a 12–14 months' storage period. *S. globosus* occurred in several instances at rather widespread brining stations, and in rather high populations (400,000 to 500,000 per ml.). In a few cases it was found to be the principal yeast responsible for the vigorous gaseous fermentation that was observed when the brine samples were collected. Although this yeast species was obtained less frequently than most of the others, it cannot be omitted from important consideration as contributing to the gaseous fermentation in certain northern brines, ranging from 25 to over 100 days in age. As indicated before, the 50 unnamed isolates of *Zygosaccharomyces* (sp. A) obtained and described by Etchells and Bell (1950 *a*) from southern fermentations are considered to be the same as the 14 isolates placed as *Z. globiformis* in the present report. In both studies, this yeast represented about the same proportion of total isolations; 3.1 and 3.5 percent respectively for the northern and southern brine yeast studies.

Yeast Sequence in the Fermentation

A general picture as to yeast sequence in the fermentation of cucumbers under northern brining conditions based on the 3-year study is presented in figure 3. In the preparation of this diagram only data regarding five of the fermentative yeast species found in the brines were used. It is our opinion that these five yeasts were the principal species that contributed most consistently to the general yeast fermentation. The lesser species, *T. caroliniana*, *S. globosus*, *Z. globiformis*, *Z.* sp. A, *Z. pastori*, and the variety of *B. sphaericus* are not shown. *Candida krusei* and *Debaryomyces membranaefaciens* var. *Holl.* were excluded on the basis

that they were only very minor species, and because they probably originated during sampling from films on vat brines and thus were not a part of the subsurface yeast fermentation.

Influence of Room Temperature Incubation on Vat Brine Samples

During late October of the 1949 season, one quart samples of cucumbers and brine were collected by the plant chemist of one of the cooperating pickle plants from 21 vats located at six of their brining stations in Indiana, Michigan, and Wisconsin. These samples were incubated at room temperature in the control laboratory of the plant for about two weeks.

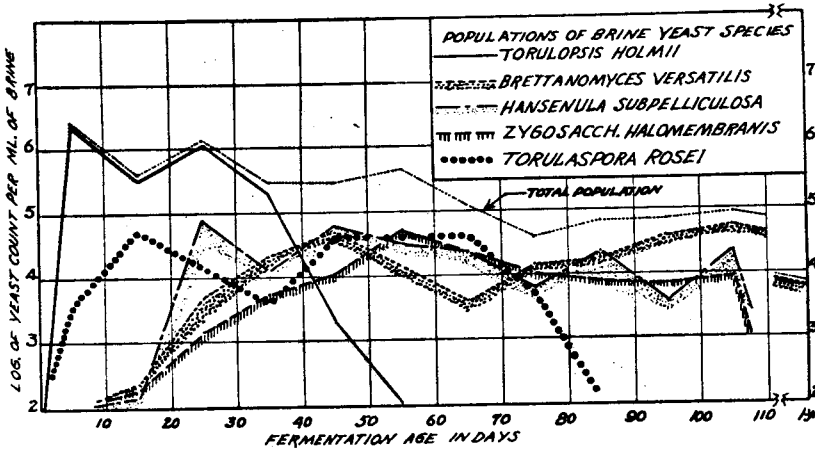

Figure 3. Yeast populations according to sequence of species in the fermentation of cucumbers brined under northern conditions.

Subsequent examination by us for the numbers and types of yeasts present is shown in table 4. Of the 55 isolates obtained, 40, or 73 percent, were *Brettanomyces versatilis*. This represents about a threefold increase in the numbers of this species compared to those obtained from brines in the same age group, but handled in the regular manner. Because of this very apparent difference in the numbers of *B. versatilis* present in the incubated brines, the 55 isolates, representing four yeast species, were not included in our results for the 1949 study. It is worth mentioning however, that five isolates of *T. rosei* were found in two of the incubated brines from one Wisconsin station. It will be recalled that this yeast was not found in the regular brines for 1949 and this was attributed in part to the fact that brining stations in Wisconsin were not visited during that season.

Pattern of Brine Yeast Species in Cucumber Fermentations

During the past five years the authors have isolated close to 1,900 yeast cultures from commercial cucumber fermentations. So far, the isolates directly related to the gaseous fermentation and subsequent storage period

TABLE 4. CLASSIFICATION OF 55 YEAST ISOLATES FROM 21 NORTHERN CUCUMBER BRINES WHICH WERE INCUBATED AT ROOM TEMPERATURE PRIOR TO EXAMINATION (1949 SEASON).

Brining Station (Code)	Vat No.	Age of Vat Brines	Chem. Exam. of Brines: Salt Conc. by Wt.	Chem. Exam. of Brines: Acidity as Lactic	Chem. Exam. of Brines: pH	Yeast Plate Count per ml. of Brine	Yeast Found and Number of Isolations: Brettanomyces *B. versatilis*	Brettanomyces *B. Sphaericus* var.	Hansenula *H. subpelliculosa*	Zygosacch. *Z. halomembranis*	Torulaspora *T. rosei*	Station Totals
		days	*percent*	*percent*		*in thous.*						
	10	71	...	...	...	28	3					
	12	71	13	0.63	3.6	...			2			
	19	77	12	.73	3.7	...	2					
	20	97	14	.54	3.7	400	2					
NJ	23	79	13	.75	3.6	140	3			2		(26)
	27	76	13	.68	3.6	...	1					
	28	76	13	.59	3.6	800	2					
	41	63	13	.66	3.8	68	3					
	43	69	14	.61	3.9	10	3					
	46	56	14	.50	4.0	710	3					
	6	76	13	0.56	3.8	260	2		1			
	26	73	13	.55	3.7	240	2					
KR	32	64	12	.52	3.6	...	2			1		
	35	57	13	.68	3.6	...		1	1			(16)
	41	79	13	.41	3.6	370	3					
	42	73	13	.32	3.8	160	2		1			
PL	27	69	...	...	...	12					2	(5)
	28	77	...	...	...	23					3	
CL	33	70	12	0.57	3.6	...	2					(2)
LA	23	76	12	0.58	3.6	28	3		1			(4)
BA	13	93	16	0.66	3.3	...	2					(2)
						Total	40	1	6	3	5	55
						% of Total	73	2	11	5	9	100%

have been classified into nine species and one variety of six genera. Based on the study of southern brines (Etchells and Bell, 1950 *a*) and on the current work, a probable pattern of brine yeast species (shown in table 5) has been prepared. From the work done to date, it becomes apparent that the pattern of species in brines from both northern and southern areas is very similar, although not necessarily identical. The occurrence of *S. globosus* in northern brines is the principal floral difference encountered to date. This overall pattern for yeasts in cucumber fermentations cer-

TABLE 5. PATTERN OF PRINCIPAL BRINE YEAST SPECIES IN COMMERCIAL CUCUMBER FERMENTATIONS.[1]

Species Found in Northern Brines	YEAST	Species Found in Southern Brines[2]
	BRETTANOMYCES	
+	*B. versatilis*	+
−	*B. sphaericus*	+
+	*B. sphaericus* var.	−
	TORULOPSIS	
+	*T. caroliniana*	+
+	*T. holmii*	+
	TORULASPORA	
+	*T. rosei*	+
	HANSENULA	
+	*H. subpelliculosa*	+
	ZYGOSACCHAROMYCES	
+	*Z. halomembranis*	+
+	*Z. globiformis*	+
	SACCHAROMYCES	
+	*S. globosus*	−

[1] Based on 1,896 isolates obtained from fermenting brines collected during five brining seasons (1946–50) from both northern and southern brining areas.

[2] Compiled from the study by Etchells and Bell (1950*a*).

tainly does not mean that additional species could not be found; the limiting factors for obtaining minor yeast species in cucumber fermentations has been discussed before (Cf. Etchells and Bell, 1950 *a*). Nor does it mean that certain species of *Candida*, *Debaryomyces*, *Rhodotorula*, and *Endomycopsis* will not occasionally be obtained from brines at the time cucumbers are brined (initial sampling), or in connection with the subsurface sampling of vats having luxuriant surface films.

SUMMARY

A study of the yeasts predominating during the fermentation of cucumbers under conditions typical of brining areas in Indiana, Michigan, and Wisconsin is presented. During three brining seasons (1948–50), 452 yeast isolates were obtained from 155 vat brines collected from 22 individual brining stations, operated by eight commercial pickle companies, in the above three states. Most of the isolations were made during a fermentation period of 2 to 105 days, although a few cultures came from vat brines that were 12 to 14 months old. During the period of observation the brines ranged from 4 to 18 percent salt by weight; 0.27 to 1.16 percent acid (calc. as lactic); and, 4.8 to 3.1 with respect to pH values.

The 452 yeast cultures were reduced to the following species listed in the order of frequency of isolation: *Brettanomyces versatilis* Etchells et Bell, 103 isolates (22.8%); *Torulaspora rosei* Guillermond, 68 (15.0%); *Torulopsis holmii* (Jörgensen) Lodder, 67 (14.8%); *Hansenula subpelliculosa* Bedford, 59 (13.1%); *Torulopsis caroliniana* Etchells et Bell, 36 (8.0%); *Brettanomyces sphaericus* var., 29 (6.4%); *Zygosaccharomyces halomembranis* Etchells et Bell, 28 (6.2%); *Saccharomyces globosus* Osterw., 22 (4.9%); *Zygosaccharomyces globiformis* Kr. et Kb., 14 (3.1%); *Candida krusei* (A. Cast.) Berkhout, 7 (1.5%); *Debaryomyces membranaefaciens* var. *Hollandicus* Lodder, 5 (1.1%); *Zygosaccharomyces* sp. A, 3 (0.7%); *Zygosaccharomyces pastori* Guillermond, 1 (0.2%); and 10 (2.2%) isolates not fully classified. The presence of a few cultures of the film yeasts *C. krusei* and *D. membranaefaciens* var. *Holl.* was attributed to heavy surface film formation on the vat brines from which subsurface brine samples were obtained.

Results on the most likely sequence of yeasts occurring during the fermentation demonstrated that two species, *Torulopsis holmii* and *Brettanomyces versatilis*, were outstanding. The first yeast predominated during the early period of fermentation (2 to 30 days) and was followed by the second yeast which was most prevalent during the late stage of fermentation (70 to 110 days), but was still present in brines after 12 to 14 months' storage. Between the above two extremes in yeast sequence, the species *Torulaspora rosei*, *Hansenula subpelliculosa*, and *Zygosaccharomyces halomembranis* were active.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the cooperation and assistance of the following pickle companies during this investigation: Squire Dingee Company, Chicago, Ill.; Budlong Pickle Company, Chicago, Ill.; Dailey Pickle and Canning Company, Saginaw, Mich.; Green Bay Foods, Green Bay, Wisc.; Libby, McNeill, and Libby, Chicago, Ill.; J. J. Gielow Sons, Detroit, Mich.; Michigan Pickle Company, Lakeview, Mich.; and H. J. Heinz Company, Pittsburgh, Pa. We also wish to express our sincere

thanks to Mr. F. O. Brown of the Squire Dingee Company for valuable assistance in collecting the brine samples throughout the study. The generous assistance of Dr. L. J. Wickerham of the Northern Regional Research Laboratory, USDA, Peoria, Ill., is also gratefully acknowledged.

REFERENCES

Bedford, C. L. 1942. A taxonomic study of the genus *Hansenula*. Mycologia, **34:** 628–649.

Bell, T. A., J. L. Etchells & I. D. Jones. 1950. Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. Food Tech., **4:** 157–163.

Custers, M. T. J. 1940. Onderzoekingen over het gistgeslacht *Brettanomyces*. Ph.D. Thesis, W. D. Meinema, Delft.

Diddens, H. A. & J. Lodder. 1942. Die Hefesammlung des "Centraalbureau voor Schimmelcultures." II. Teil. Die anaskosporogenen Hefen. 2 Hft. Amsterdam.

Etchells, J. L. 1941. Incidence of yeasts in cucumber fermentations. Food Research, **6:** 95–104.

Etchells, J. L. & T. A. Bell. 1950 *a*. Classification of yeasts from the fermentation of commercially brined cucumbers. Farlowia, **4:** 87–112.

Etchells, J. L. & T. A. Bell. 1950 *b*. Film yeasts on commercial cucumber brines. Food Tech., **4:** 77–83.

Etchells, J. L. & T. A. Bell. 1951. Pectin hydrolysis by yeasts from commercial cucumber brines and related sources. Unpublished.

Etchells, J. L. & I. D. Jones. 1946. Procedure for bacteriological examination of brined, salted, and pickled vegetables and vegetable products. Amer. J. Pub. Health, **36:** 1112–1123.

Hall, H. H. & Dorothea Teunisson. 1948. *Personal communications.*

Lodder, J. 1934. Die Hefesammlung des "Centraalbureau voor Schimmelcultures." II. Teil. Die anaskosporogenen Hefen. 1 Hft. Amsterdam.

Luh, B. S. & H. J. Phaff. 1948. Pectin breakdown by yeasts. Fruit Prod. J. and Amer. Food Mfgr., **28:** 118. (Abstract.)

Stelling-Dekker, N. M. 1931. Die Hefesammlung des "Centraalbureau voor Schimmelcultures." I. Teil. Die sporogenen Hefen. Amsterdam.

Wickerham, L. J. 1946. A critical evaluation of the nitrogen assimilation tests used in the classification of yeasts. J. Bact., **52:** 293–301.

Wickerham, L. J. 1951. Taxonomy of yeasts. U. S. Dept. Agr. Tech. Bull. No. 1029, 1–56.