# Influence of Sorbic Acid on Populations and Species of Yeasts Occurring in Cucumber Fermentations[1]

JOHN L. ETCHELLS, ALFRED F. BORG,[2] AND THOMAS A. BELL

*U. S. Food Fermentation Laboratory,[3] North Carolina State College, and Departments of Botany and Animal Industry, North Carolina Agricultural Experiment Station, Raleigh, North Carolina*

Received for publication June 15, 1960

In the natural fermentation of brined cucumbers a part of the subsurface microbial activity is due to yeasts. Active growth by these organisms in commercial cucumber brines was first reported in 1941 (Etchells). Subsequent work on this phase of the fermentation has included studies directed toward establishing the identity of the individual species comprising the total yeast population in brines. Information of this type has been reported for two major cucumber brining areas in the country—northern and southern (Etchells and Bell, 1950; Etchells, Costilow, and Bell, 1952). The findings of these two studies, based on the identity of nearly 1900 cultures of yeasts isolated from brines during various stages of fermentation, revealed that the pattern for the principal yeast species in brines from both brining areas was very similar. Seven of the nine species found were obtained from both northern and southern brines.

During the brining seasons of 1954, 1955, and 1956 we had an opportunity to extend that portion of the work related to the southern brining areas while studying the microbial flora in experimental cucumber brines to which sorbic acid (2,4-hexadienoic acid) or its sodium salt had been added. This fungistat was introduced into the brine in an attempt to control yeasts and thus prevent the formation of "bloaters" (hollow cucumbers) caused by gaseous fermentation (Jones *et al.*, 1941). A number of yeasts were isolated from brines both with and without sorbic acid. It is our purpose in this paper to list the species isolated under the various experimental conditions used and to compare these findings with those previously reported from this laboratory.

## MATERIALS AND METHODS

The cucumbers were brined in wooden vats each containing approximately 30 bushels of freshly harvested no. 3 size Model variety pickling stock. The vats were unsheltered and located under outside conditions at a commercial pickling plant in eastern North Carolina. Each vat, after being filled with cucumbers, representing a composite of the stock from two or more receiving stations, was fitted with a false, wooden head and salt brine of the desired concentration added to a level of a few inches above the head. Next, dry salt, in appropriate amounts, was added on the false head to maintain the desired initial brine concentration which otherwise would become diluted by the water content of the cucumbers. Sorbic acid or sodium sorbate was added to give the desired concentration by weight

[1] Published with the approval of the Director of Research, North Carolina Agricultural Experiment Station, as paper no. 1172 of the Journal Series.

[2] Present address: Department of Bacteriology, Kansas State University, Manhattan, Kansas.

[3] One of the laboratories of the Southern Utilization Research Development Division, Agricultural Research Service, U. S. Department of Agriculture.

on the basis of the total content of vat; namely, the weight of cucumbers plus the weight of the water in the brine. Brine temperatures during the active fermentation period were in the range of 25 to 30 C.

During the three brining seasons (1954 to 1956), 718 yeast cultures were obtained from 60 experimental fermentations, 35 of which contained sorbic acid. Information on the source of these cultures with respect to the various brining treatments employed during the 3-year period, together with details on the rate of increase of the initial brine strength for each treatment, is given in table 1. Also, a general picture with respect to yeast activity in the brining treatments examined during the investigation is included in this table.

The brine samples were collected, plated, and counted according to the methods previously described by Etchells and Jones (1946) and revised by Etchells and Bell (1950). The methods and classification systems employed for the 718 isolates were essentially those of the Dutch workers Lodder and Kreger-van Rij (1952); the earlier work by Stelling-Dekker (1931) was followed in some instances. The classification system of Wickerham (1951) was used for the genus *Hansenula*. Certain other techniques employed in connection with the taxonomic and isolation procedures have been fully described before (Etchells and Bell, 1950; Etchells, Costilow, and Bell, 1952).

## RESULTS

The experimental conditions used in each of the three brining seasons and their effects on the mean yeast populations are summarized in table 1. In 1954, the yeast population in control brines at 15.8 per cent dropped considerably as compared to the lower salt treatment of 11.9 per cent. No such difference in population density was noted between control brines containing 9.2 and 11.9 per cent salt. In the presence of 0.10 per cent sorbic acid, yeast growth was almost entirely suppressed at all the brine strengths used.

Comparable results were obtained in 1955, where only one brine strength was used and three concentrations of sorbic acid, namely 0.025, 0.05, and 0.10 per cent. The mean population of the controls was much less than in the preceding season, but the strong inhibitory effect of sorbic acid was still evident. Even the lowest concentration of sorbic acid kept the yeast population to less than 1000 per ml of brine.

In 1956, 0.025 per cent sorbic acid again effectively controlled the development of yeasts. The low yeast populations in control vats can probably be attributed to lack of brine nutrients since a vigorous lactic fermen-

TABLE 1

*Origin of 718 yeast isolates from 826 brine samples obtained from 60 cucumber fermentations located in eastern North Carolina (1954 to 1956 brining seasons)*

| Season | Brining Treatment* | | | | Yeast Populations in Brine | | Yeast Isolates Obtained§ |
|---|---|---|---|---|---|---|---|
| | No. of vats† | Initial salt concentration of cover brine | | Sorbic acid added‡ | Range | Mean | |
| | | °sal | % | % | *per ml* | *per ml* | *no.* |
| 1954 | 4 | 35 | 9.2 | 0 | 13,000–10,000,000 | 1,250,000 | 84 |
| | 4 | 35 | 9.2 | 0.10 | 1–100 | 54 | 30 |
| | 2 | 45 | 11.9 | 0 | 23,000–1,950,000 | 1,550,000 | 32 |
| | 2 | 45 | 11.9 | 0.10 | 2–40 | 15 | 16 |
| | 1 | 60 | 15.8 | 0 | 126,000–1,200,000 | 350,000 | 15 |
| | 1 | 60 | 15.8 | 0.10 | 20–300 | 275 | 9 |
| 1955 | 8 | 35 | 9.2 | 0 | 10–2,100,000 | 190,000 | 159 |
| | 8 | 35 | 9.2 | 0.025 | 10–8,000 | 920 | 66 |
| | 8 | 35 | 9.2 | 0.05 | 10–4,100 | 305 | 18 |
| | 2 | 35 | 9.2 | 0.10 | 10–200 | 105 | 2 |
| 1956 | 10 | 17 | 4.5 | 0 | 250–225,000 | 59,000 | 135 |
| | 10 | 17 | 4.5 | 0.025 | 12–7,600 | 1,380 | 152 |
| Total..... | 60 | | | | | | 718 |

* The 9.2 and 11.9 per cent salt brine treatments were maintained at those concentrations for the first week and then increased 5° salometer (1° sal = 0.264 per cent salt) per week to 60° sal; the 15.8 per cent treatment was maintained at that concentration throughout; the 4.5% treatment was maintained at that concentration for 10 days then raised 10° sal per week to 60° sal.

† The vats were located outside, unsheltered, and each contained approximately 30 bushels of no. 3 size (from 1½ to 2 in. diameter) Model variety cucumbers.

‡ During 1955 and 1956 seasons, sodium sorbate was added to give an equivalent amount of sorbic acid.

§ Fermentation period covered by isolations: 1954, 19 to 112 days; 1955, 0 to 48 days; 1956, 10 hr to 60 days.

tation took place at this weak brine strength (4.5 per cent salt) prior to development of the yeast population.

The effect of brine strength and of sorbic acid on the composition of the yeast flora during the 1954, 1955, and 1956 seasons is shown in table 2. The results for individual treatments during the first two seasons have been combined for each year and presented on a seasonal basis. This was done because, with one or two exceptions noted in the text, the findings were wholly comparable. Of the 133 isolates obtained from control

TABLE 2

*Classification of 718 yeast isolates obtained from cucumber fermentations with and without sorbic acid (SA) during three brining seasons*

| Yeast | Isolates Obtained from Fermentations | | | | | | | Presence (+) or Absence (−) of Species in | |
|---|---|---|---|---|---|---|---|---|---|
| | 1954 Season | | 1955 Season | | 1956 Season | | Total isolations | Control brines | SA brines |
| | Control brines | SA brines | Control brines | SA brines | Control brines | SA brines | | | |
| | no. | no. | no. | no. | no. | no. | no. | | |
| *Brettanomyces* | | | | | | | | | |
| *B. versatilis** | 51 | 8 | 61 | 1 | 0 | 0 | 121 | + | + |
| *Candida* | | | | | | | | | |
| *C. guilliermondii* | 0 | 0 | 0 | 0 | 1 | 1 | 2 | + | + |
| *C. krusei** | 0 | 0 | 0 | 0 | 98 | 104 | 202 | + | + |
| *C. parapsilosis* | 0 | 0 | 4 | 3 | 0 | 0 | 7 | + | + |
| *C. tropicalis* | 0 | 0 | 0 | 8 | 16 | 34 | 58 | + | + |
| *Debaryomyces* | | | | | | | | | |
| *D. globosus* | 0 | 0 | 1 | 0 | 0 | 0 | 1 | + | − |
| *D. kloeckera* | 0 | 12 | 0 | 0 | 0 | 0 | 12 | − | + |
| *D. nicotianae** | 0 | 0 | 0 | 2 | 0 | 0 | 2 | − | + |
| *Hanseniaspora* | | | | | | | | | |
| *H. valbyensis* | 0 | 0 | 1 | 1 | 9 | 2 | 13 | + | + |
| *Hansenula* | | | | | | | | | |
| *H. anomala* | 0 | 1 | 15 | 2 | 0 | 0 | 18 | + | + |
| *H. subpelliculosa** | 17 | 5 | 37 | 2 | 0 | 0 | 61 | + | + |
| *Kloeckera* | | | | | | | | | |
| *K. apiculata** | 0 | 0 | 0 | 1 | 5 | 2 | 8 | + | + |
| *Pichia* | | | | | | | | | |
| *P. polymorpha* | 0 | 0 | 0 | 0 | 0 | 1 | 1 | − | + |
| *Rhodotorula* | | | | | | | | | |
| *R. flava* | 0 | 0 | 0 | 0 | 0 | 1 | 1 | − | + |
| *R. glutinis* | 0 | 0 | 7 | 11 | 0 | 0 | 18† | + | + |
| *Rhodotorula* sp. | 0 | 0 | 1 | 0 | 0 | 0 | 1 | + | − |
| *Saccharomyces* | | | | | | | | | |
| *S. cerevisiae* | 0 | 0 | 0 | 0 | 1 | 3 | 4 | + | + |
| *S. elegans** | 1 | 10 | 1 | 9 | 0 | 0 | 21 | + | + |
| *S. exiguus* | 0 | 0 | 5 | 0 | 0 | 0 | 5 | + | − |
| *S. mellis* | 0 | 0 | 5 | 25 | 0 | 0 | 30 | + | + |
| *S. rosei** | 1 | 7 | 14 | 8 | 0 | 0 | 30 | + | + |
| *Torulopsis* | | | | | | | | | |
| *T. candida* | 0 | 0 | 0 | 3 | 0 | 0 | 3 | − | + |
| *T. caroliniana** | 50 | 9 | 0 | 0 | 0 | 0 | 59 | + | + |
| *T. famata* | 0 | 0 | 0 | 3 | 0 | 0 | 3 | − | + |
| *T. glabrata* | 8 | 0 | 1 | 1 | 5 | 4 | 19 | + | + |
| *T. gropengiesseri* | 0 | 0 | 2 | 3 | 0 | 0 | 5 | + | + |
| *T. magnoliae* | 0 | 0 | 0 | 1 | 0 | 0 | 1 | − | + |
| *T. molischiana* | 0 | 0 | 3 | 0 | 0 | 0 | 3 | + | − |
| *Torulopsis* sp. | 3 | 3 | 1 | 2 | 0 | 0 | 9 | + | − |
| Subtotal | 131 | 55 | 159 | 86 | 135 | 152 | | | |
| Total | 186 | | 245 | | 287 | | 718 | | |

* Species previously found in the subsurface yeast flora of cucumber brines (Costilow and Fabian, 1953; Etchells and Bell, 1950; Etchells *et al.*, 1952, 1953).

† Only two isolates—both *Rhodotorula glutinis*—were obtained from the fermentations in 1955 containing 0.10 per cent sorbic acid; these are included in the total for this species.

brines in 1954, 118 were placed in 3 species; *Torulopsis caroliniana* (50 isolates), *Brettanomyces versatilis* (51), and *Hansenula subpelliculosa* (17). The remaining 15 isolates comprised 14 cultures representing 4 other species (all isolated from the 9.2 per cent brines). Essentially the same group of yeasts was isolated from fermentations containing 0.10 per cent sorbic acid. However, the isolates from sorbic acid vats were not predominantly of any one species or group of species, but were distributed fairly evenly through 8 species. The 12 isolates of *Debaryomyces kloeckera* all came from sorbic acid treated brines.

The fermentations examined during the 1955 season yielded a much broader spectrum of species of yeasts than did the 1954 brines (table 2). A total of 241 cultures representing 20 species in 9 genera were identified. Four other cultures were identified as to genus only. Fourteen of the species were found in the control vats, and 2 species, namely *Saccharomyces exiguus* (5 isolates) and *Debaryomyces globosus* (1), were obtained from control vats only. Six species represented by 18 isolates were found only in vats containing sorbic acid. The two species isolated with greatest frequency from control vats, *i.e.*, *B. versatilis* (61 isolates) and *H. subpelliculosa* (37), were almost entirely absent from fermentations containing sorbic acid. Only one culture of the former species and two of the latter were recovered from sorbic acid brines and only at the lowest concentration used. *T. caroliniana* was strangely absent from all fermentations even though conditions appeared to be essentially the same as for the 1954 season where it was isolated from all brining treatments.

The dominant species found during the 1954 and 1955 seasons appeared to be much the same regardless of the salt concentrations employed. However, the addition of sorbic acid altered the picture markedly as noted for *B. versatilis* and *H. subpelliculosa* in the 1955 season.

The experiments during the 1956 season were carried out at the relatively low brine strength of 4.5 per cent salt. Studies on the composition of the subsurface yeast flora from fermenting cucumber brines in this general range of salt concentration have not been reported heretofore. It is of interest, therefore, to note that the predominant yeasts were not the same as those observed in the two preceding seasons.

Two species of *Candida*, namely, *Candida krusei* and *Candida tropicalis*, dominated the fermentation (table 2) and comprised 252 of the 287 isolates obtained in 1956. Each species was recovered about as frequently from the sorbic acid treated brines as it was from the control brines. Of the seven additional species isolated, five were obtained from both the control brines and those containing sorbic acid; single isolates of two species came only from sorbic acid brines. The two predominating species found (*C. krusei* and *C. tropicalis*) might normally be considered part of the surface yeast flora responsible for film formation on low salt content cucumber brines sheltered from direct sunlight. However, in the present instance the vats were in the open and the brine surface in each case was completely free of film yeast development. Thus, the principal subsurface yeast activity in the 4.5 per cent brines, especially the controls, must be attributed to these two species of *Candida*.

It is apparent from the yeast populations shown in table 1 that the addition of sorbic acid to brines drastically suppressed but did not eliminate these organisms. These findings are in general agreement with those of Costilow *et al.* (1957), and particularly so for that portion of their work (1954 season) in which the sorbic acid added to brines was calculated on a basis comparable to that given in the present study.

Summarized results presented in table 2 denoting presence or absence of individual species in control and sorbic acid treated brines demonstrated that the pattern of species occurrence under both conditions (with or without sorbic acid) can be most similar. Of the 27 species listed, 17 were obtained from both control and sorbic acid brines; 3 species were obtained only from control brines; and 7 species were obtained only from sorbic acid treated brines. Four of the seven species in the latter category were represented by single isolations; two other species were represented by only three isolates each. Among the three yeast species found only in the control brines, one species was represented by a single isolate, another by three isolates and the third by five isolates. Further study undoubtedly would show that many of the species obtained exclusively in the absence or presence of sorbic acid could be obtained from the opposite conditions. For example, *Torulopsis glabrata*, isolated only from control vats in 1954, was obtained from both control and sorbic acid brines in 1955 and 1956.

Eight of the 27 species listed in table 2 have been denoted as having been previously reported as occurring in the subsurface yeast flora of commercial cucumber brines. The remaining 19 species listed are those which have not been previously reported, to our knowledge, from cucumber fermentations.

## Discussion

During the past 10 years, identification studies have been made on almost 3000 cultures of yeasts isolated from commercial cucumber fermentations located in the principal brining areas of the country—northern, northwestern, and southern (Etchells and Bell, 1950; Etchells *et al.*, 1952; Costilow and Fabian, 1953; Etchells, Bell, Lewis, and Ijichi, 1956, Unpublished Experiments). After a review of the taxonomic findings cited above and in the present report, it becomes evident that certain genera and species appear to

dominate the subsurface yeast phase of commercial cucumber fermentations with initial brine strengths of 7 to 10 per cent salt and raised gradually to 16 per cent. Furthermore, their pattern of occurrence in brines from different areas of the country may be very similar although not necessarily identical. It seems likely that the following 10 species, listed in the approximate order of their frequency of occurrence, might be expected to be isolated from the active fermentation and subsequent storage period of commercially brined cucumbers: *Brettanomyces versatilis*, *Hansenula subpelliculosa*, *Torulopsis caroliniana*, *Torulopsis holmii*, *Saccharomyces rosei*, *Saccharomyces halomembranis*, *Saccharomyces elegans*, *Saccharomyces delbrueckii*, *Brettanomyces sphaericus*, and *Hansenula anomala*. This constitutes a revision of the species pattern first proposed by Etchells *et al.* (1952).

The subsurface yeast flora of cucumber fermentations at brine strengths significantly lower than those used in commercial practice is worth special note. It will be recalled that two species, *C. krusei* and *C. tropicalis*, dominated the yeast flora in the weak brines examined during the 1956 season. We suggest that the low salt concentration of the brine employed (4.5 per cent) was the ecological factor mainly responsible for these two species being able to outgrow species of *Brettanomyces*, *Hansenula*, *Torulopsis*, and *Saccharomyces* normally found in abundance at the higher brine strengths. The relatively low salt tolerance for *C. krusei* has been established earlier (Etchells, Bell, and Jones, 1953). Also, this species was considered by Mrak *et al.* (1956) to be the principal yeast present in two commercial olive brines during the fermentative period. The salt content of the olive brines was maintained at 6.5 per cent throughout, which is well within the tolerance for good growth by *C. krusei* and only slightly higher initially than the 4.5 per cent brine treatment used in the current study.

There is no clear-cut explanation for the fact that some species were found only when sorbic acid was absent, and others only when it was present. Isolation of but a single culture or very few cultures of a given species under specific conditions can well be attributed to chance. The limiting factors for obtaining minor yeast species from commercial cucumber fermentations have been discussed before (Etchells and Bell, 1950). It is possible that sorbic acid, by repressing the normally dominant population, allows one to isolate the minor types which ordinarily are present but not obtainable by conventional procedures. The possibility that the distribution of such minor types may be due to their susceptibility or resistance to sorbic acid has not been explored in the present investigation.

However, Costilow, Ferguson, and Ray (1955) and Bell, Etchells, and Borg (1959) have studied the influence of sorbic acid on the growth of a number of species of yeasts previously isolated from commercial cucumber fermentations. Their results revealed that a considerable difference can be expected between species as to their growth response in the presence of sorbic acid in concentrations from 0.010 to 0.050 per cent. Both groups of workers found *C. krusei* to be among the limited number of yeast species that were most tolerant to sorbic acid. This may in part be responsible for the predominance of this species during the 1956 season in in the low salt content brines (4.5 per cent) containing sorbic acid.

Changes in conditions of brining such as alteration of the brine strength can alter not only the total yeast population but the number of species comprising the population. This was demonstrated earlier under commercial conditions (Etchells and Bell, 1950) as well as in the present experiments. However, changing the brining process by use of salt alone usually influences the start and duration of yeast activity in brines (Etchells, 1941; Etchells and Jones, 1943) but has limited use as method for adequately controlling this group of organisms and the type of spoilage (bloaters) attributed to them (Jones *et al.*, 1941; Etchells *et al.*, 1953). The use of sorbic acid effectively controlled the development of yeasts in the brining studies reported herein. However, essentially the same species survived in the sorbic acid treated brines as were found in the controls.

It is of interest to speculate on the possibility of the surviving yeasts developing sufficient tolerance to sorbic acid to produce increasingly higher populations in brines over a period of several seasons. This supposition becomes more interesting when one considers that the initial amount of sorbic acid added to cucumber brines does not remain constant but actually undergoes a progressive loss during the fermentation period (Costilow *et al.*, 1957; Alderton and Lewis, 1958). Further, the rate of loss of the chemical from brine fermentations appears to be related to the salt concentration employed. For example, in 4.5 per cent salt brines containing 0.025 per cent sorbic acid at the start, the decrease was found to be 24, 50, 72, and 90 per cent after 5, 10, 30, and 60 days, respectively; however, in 9.2 per cent salt brines, with the same concentration of the chemical, the loss was more gradual, *i.e.*, 20, 40, and 60 per cent after 10, 30, and 100 days, respectively (Bell and Etchells, 1956, Unpublished Experiments). Such conditions, in time, would seem to offer ample opportunity for the development of a yeast flora with a high degree of tolerance to sorbic acid.

## Acknowledgments

The authors gratefully acknowledge the cooperation, assistance, and facilities provided throughout the study by Lutz and Schramm Inc., Ayden, North Carolina. We are particularly grateful to Mr. W. G. Griffith of

that company for his fine contribution in brining the cucumbers and collecting the samples during the investigation.

## SUMMARY

Results of a 3-year study on the influence of sorbic acid on populations and species of yeasts occurring in cucumber fermentations brined at a commercial pickling plant located in eastern North Carolina are presented. During three brining seasons (1954 to 1956), 718 yeast isolates were obtained by frequent sampling of 60 experimental fermentations representing four brining treatments (4.5, 9.2, 11.9, and 15.8 per cent salt) with or without the addition of sorbic acid (0.025, 0.050, and 0.10 per cent).

The yeast cultures were reduced to the following 10 genera listed in the order of frequency of isolation: *Candida*, 269 isolates (4 species); *Brettanomyces*, 121 (1); *Torulopsis*, 102 (7); *Saccharomyces*, 90 (5); *Hansenula*, 79 (2); *Rhodotorula*, 20 (2); *Debaryomyces*, 15 (3); *Hanseniaspora*, 13 (1); *Kloeckera*, 8 (1); and *Pichia*, 1 (1).

The addition of sorbic acid drastically suppressed yeast populations in fermentations at the various brine concentrations employed during the investigation. However, the chemical did not eliminate these organisms. Furthermore, the results revealed that the pattern of species occurrence with or without sorbic acid can be qualitatively similar.

The principal subsurface yeast activity in the fermentations at low brine strength (4.5 per cent salt) was attributed to two species of *Candida*, namely *Candida krusei* and *Candida tropicalis*. This finding represented a marked change in flora compared to that observed at substantially higher brine strengths (9.2 to 15.8 per cent salt) where four different yeasts—*Brettanomyces versatilis*, *Hansenula subpelliculosa*, *Torulopsis caroliniana* and *Saccharomyces rosei*—were considered to be the dominant species.

## REFERENCES

ALDERTON, G. AND LEWIS, J. C. 1958 Determination of sorbic acid and its disappearance from pickle brines. Food Research, **23**, 338–344.

BELL, T. A., ETCHELLS, J. L., AND BORG, A. F. 1959 Influence of sorbic acid on the growth of certain species of bacteria, yeasts, and filamentous fungi. J. Bacteriol., **77**, 573–580.

COSTILOW, R. N. AND FABIAN, F. W. 1953 Microbiological studies of cucumber fermentations. Appl. Microbiol., **1**, 314–319.

COSTILOW, R. N., FERGUSON, W. E., AND RAY, S. 1955 Sorbic acid as a selective agent in cucumber fermentations. I. Effect of sorbic acid on microorganisms associated with cucumber fermentations. Appl. Microbiol., **3**, 341–345.

COSTILOW, R. N., COUGHLIN, F. M., ROBBINS, E. K., AND HSU, WEN-TAH 1957 Sorbic acid as a selective agent in cucumber fermentations. II. Effect of sorbic acid on the yeast and lactic acid fermentations in brined cucumbers. Appl. Microbiol., **5**, 373–379.

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

ETCHELLS, J. L. AND JONES, I. D. 1943 Bacteriological changes in cucumber fermentations. Food Inds., **15**(2), 54–56.

ETCHELLS, J. L. AND JONES, I. D. 1946 Procedure for bacteriological examination of brined, salted, and pickled vegetables and vegetable products. Am. J. Public Health, **36**, 1112–1123.

ETCHELLS, J. L. AND BELL, T. A. 1950 Classification of yeasts from the fermentation of commercially brined cucumbers. Farlowia, **4**, 87–112.

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., BELL, T. A., AND JONES, I. D. 1953 Morphology and pigmentation of certain yeasts from brines and the cucumber plant. Farlowia, **4**, 265–304.

JONES, I. D., ETCHELLS, J. L., VEERHOFF, O., AND VELDHUIS, M. K. 1941 Observations on bloater formation in cucumber fermentation. Fruit Prod. J., **20**, 202–206; 219, 220.

LODDER, J. AND KREGER-VAN RIJ, N. J. W. 1952 *The yeasts. A taxonomic study.* North Holland Publishing Co., Amsterdam.

MRAK, E. M., VAUGHN, R. H., MILLER, M. W., AND PHAFF, H. J. 1956 Yeasts occurring in brines during the fermentation and storage of green olives. Food Technol., **10**, 416–419.

STELLING-DEKKER, N. M. 1931 Die Hefesammlung des Centraalbureau voor Schimmelcultures. I. Teil. Die sporogenen Hefen. Verhandel. Koninkl. Akad. Wetenschap. Amsterdam, Afdeel. Natuurk., Sect. II, **28**, 1–547.

WICKERHAM, L. J. 1951 Taxonomy of yeasts. U. S. Dept. Agr. Tech. Bull. No. 1029, 56 pp.