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Film Yeasts on Commercial Cucumber Brines[a, b]

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A study of the types of film yeasts found on commercial cucumber brines, ranging in salt content from 5 to 19 percent salt by weight is presented. The cultural and biochemical properties for the species placed in 4 genera are given.

The surface of cucumber brines sheltered from direct sunlight develops luxuriant films attributed to film-forming yeasts. The matter of film yeast control has been a controversial question in the industry. Some operators prefer to remove the film by skimming; some to keep the brine surface stirred to discourage film formation; others to leave the film growth undisturbed. Before adequate control measures can be considered, more information on the identity of the common types occurring on cucumber brines, and their biochemical properties, is necessary.

The present investigation was undertaken to obtain some of the specific information needed on the principal types of these film-forming yeasts. The experiments were conducted under conditions typical of the industry in several sections of the United States.

Recently, Etchells and Bell *(3)* have stressed the need for a clearcut distinction to be made between the surface yeasts and subsurface yeasts in relation to cucumber brines, a point frequently confused in the literature. These authors obtained and identified 1,424 isolates of the latter type from 42 actively fermenting brines. The cultures were reduced to the following six genera in the order of frequency of isolation: *Torulopsis,* 721 isolates; *Brettanomyces,* 588; *Zygosaccharomyces,* 59; *Hansenula,* 49; *Torulaspora,* 6; and *Kloeckera,* 1. Species of film yeasts such as found by Mrak and Bonar *(7)* on brined foods were not obtained.

Source of Brines

In the present work, 47 cultures from commercial brines were collected during the 1947 and 1948 brining seasons. These cultures came from the surface films of brines ranging in salt content from 5 to 19 percent salt by weight and represented 40 commercial vat brines from 12 brining stations in the following geographical areas: North Carolina, 14 vats; Wisconsin, 12; Indiana, 6; Georgia, 5; and Michigan, 3. The origin and generic classification of yeasts on these brines are shown in Table 1.

An additional group of 14 isolates of *Endomycopsis* were obtained from cucumbers brined under laboratory conditions at 5 to 6 percent salt.

Previous Investigations

Mrak and Bonar *(7)* investigated 28 cultures isolated from the surface films of 27 samples of various brined and pickled products (dill pickles, cucumber salt-stock, Zucca melon, green olives, Sicilian olives, dill weed, cauliflower, and ham). They found film yeasts that belonged to species of three genera: *Debaryomyces,* 16 isolates; *Pichia,* 9; *Mycoderma,* 3. The *Debaryomyces* were found to be very salt-tolerant and predominated on brines of 15 percent and above. The other two genera were found on the brines ranging from 4 to 15 percent. Of particular interest to the present study is the finding of these workers that *Debaryomyces* was responsible for the films on all 7 samples of salt-stock cucumbers they examined. Further, of the 7 isolates they obtained from the films on 7 samples of dill pickles, 5 were placed as *Pichia* and 2 as *Mycoderma.*

Graham and Hastings *(5)* isolated 6 cultures of film-forming yeasts from the surface of rennet brines; all were placed in two species of *Debaryomyces.* Diddens and Lodder *(2)* listed salt pickles as the source of 5 cultures of *Candida krusei* but did not mention whether they came from films on the brine.

Review articles on yeasts appearing in 1941 *(6),* 1947 *(9),* and 1948 *(8)* list no additional genera occurring as films on brined or salted vegetables other than those that have been mentioned. The earlier literature on film-forming yeasts on brines, up to 1939, dealt chiefly with the occurrence of the genus *Mycoderma.* This has been adequately reviewed by Mrak and Bonar *(7)* as well as information on the salt-tolerance of yeasts in general.

Procedure

The cultures were taken directly from surface films on the brines and streaked onto previously poured plates of acidified, glucose agar *(4)* containing 6 to 8 percent salt by weight. Well-isolated colonies were then restreaked on the same medium for purification. The taxonomic methods employed and classification systems used were essentially those outlined by the Dutch workers *(1, 2, 10).* Certain recently described *(3)* modifications and additions to their techniques were included. For salt tolerance tests, a liquid medium was used consisting of cucumber brine fortified by the addition of glucose and ethyl alcohol in 1.0 percent amounts. The salt concentration of the brine was adjusted to cover a range from 5 to 20 percent by weight. To facilitate comparison of film formation by different yeasts, petri dishes with the bottoms divided into 3 and 4 sections were used (see Figure 1).

Genera Found

The 47 cultures were placed in the following genera: *Debaryomyces,* 22 isolates; *Endomycopsis,* 12; *Zygosaccharomyces,* 9; and, *Candida,* 4. An additional 14 isolates of *Endomycopsis* were obtained from a like number of films on cucumbers brined under laboratory conditions and were considered a different variety than those found on commercial brines. Yeasts belonging to

[a] Agricultural Chemical Research Division Contribution No. 247.

[b] This study was carried out under a cooperative project with the Department of Horticulture of the North Carolina Agricultural Experiment Station.

TABLE 1

Origin and Generic Classification of 47 Film-Forming Yeasts Occurring on 40 Commercial Cucumber Brines and Obtained from 12 Brining Stations Located in Five States

State	Brining Stations and Individual Vat Films Sampled		Age of Vat Brines at Sampling	Cultures Obtained from Films		Chemical Examination of Brines		
				Code	Genera [e]	Salt Conc.	Acidity as Lactic	pH
	Sta.	*Film No.*	*days*			*percent*	*percent*	
North Carolina (4 Brining Stations; 20 Isolates)	A	1	5	FY-24	Endomycopsis	6	....	....
		2	7	29	Endomycopsis	9	....	....
		3	13	25	Endomycopsis	13	....	....
		4	13	26	Endomycopsis	13	....	....
		5	13	27	Endomycopsis	13	....	....
		6	15	30	Endomycopsis	14	....	....
		7	20	28	Endomycopsis	15	....	....
	B	8 [d]	12	Y-470	Endomycopsis	13	0.25	4.3
			12	474	Endomycopsis	13	0.25	4.3
			12	475	Endomycopsis	13	0.25	4.3
			18	FY-15	Endomycopsis	15	0.32	3.7
			22	16	Endomycopsis	15	0.30	3.8
			22	16R	Debaryomyces	15	0.30	3.8
	C	9 [e]	12	FY-17	Candida	5	0.70	3.4
			12	18	Candida	5		
		10 [e]	14	19	Candida	5	0.70	3.4
			14	20	Candida	5		
	D	11	20	FY-21	Debaryomyces	15	....	....
		12	20	22	Debaryomyces	15	....	....
		13	20	23	Debaryomyces	15	....	....
Wisconsin (4 Brining Stations; 12 Isolates)	A	14	35	NFY-21	Debaryomyces	11	0.74	....
		15	63	10	Debaryomyces	12	0.72	3.4
		16	72	24	Debaryomyces	13	0.70	3.4
		17	80	25	Debaryomyces	12	0.88	3.5
		18	1-year	22	Debaryomyces	16	0.55	3.6
	B	19	48	NFY-19	Debaryomyces	15	0.58	3.4
		20	69	3	Debaryomyces	14	0.71	3.5
		21	76	20	Debaryomyces	14	0.75	3.4
		22	92	1	Zygosaccharomyces	15	0.70	3.5
	C	23	85	NFY-13	Debaryomyces	13	0.94	3.2
		24	99	12	Debaryomyces	13	0.82	3.3
	D	25	67	NFY-26	Zygosaccharomyces	19	0.35	3.6
Indiana (2 Brining Stations; 6 Isolates)	A	26	54	NFY-23	Zygosaccharomyces	16	0.59	....
		27	62	7B	Zygosaccharomyces	13	0.85	3.7
		28	68	9	Zygosaccharomyces	15	0.54	3.8
		29	83	6	Zygosaccharomyces	15	0.74	3.7
		30	1-year	8	Zygosaccharomyces	16	0.46	3.9
	B	31	58	NFY-18	Debaryomyces	13	0.64	3.5
Michigan (1 Brining Station; 4 Isolates)	A	32	39	NFY-15	Debaryomyces	12	0.50	....
		33	50	14	Debaryomyces	13	0.82	3.6
		34	72	16	Zygosaccharomyces	13	0.72	3.5
		35	72	16B	Zygosaccharomyces	13	0.72	3.5
Georgia (1 Brining Station; 5 Isolates)	A	36	39	FY-33	Debaryomyces	12	....	....
		37	39	36	Debaryomyces	12	....	....
		38	39	37	Debaryomyces	12	....	....
		39	36	34	Debaryomyces	12	....	....
		40	36	35	Debaryomyces	12	....	....

[e] Does not include 14 isolates of *Endomycopsis* from films on 14 one-gallon lots of salt-stock cucumbers brined under laboratory conditions at 5-6 percent salt concentration.

[d] Six cultures isolated from film at 3 different time intervals.

[e] Two cultures isolated from film.

TABLE 2

Distribution of Film-Forming Yeasts Occurring on Cucumber Brines According to States

Yeast	Number of Isolates [f]	Distribution as to Brining Area:				
		North Carolina	Wisconsin	Indiana	Georgia	Michigan
Debaryomyces	22					
D. membranaefaciens var. *Hollandicus*		4	9	1	2	2
D. species (smooth)		0	1	0	3	0
Endomycopsis	26					
E. ohmeri		12	0	0	0	0
E. ohmeri var. *minor* [g]		14				
Zygosaccharomyces	9					
Z. halomembranis		0	2	5	0	2
Candida	4					
C. krusei		4	0	0	0	0

[f] The 61 isolates represent two groups; 47 came from films sampled from 40 commercial vat brines (N. C. 14 vats; Wisc. 12; Ind. 6; Ga. 5, and Mich. 3), and 14 came from films on 1-gal. lots of salt-stock brined under laboratory conditions at about 5-percent salt.

[g] The 14 isolates of this species came from laboratory source.

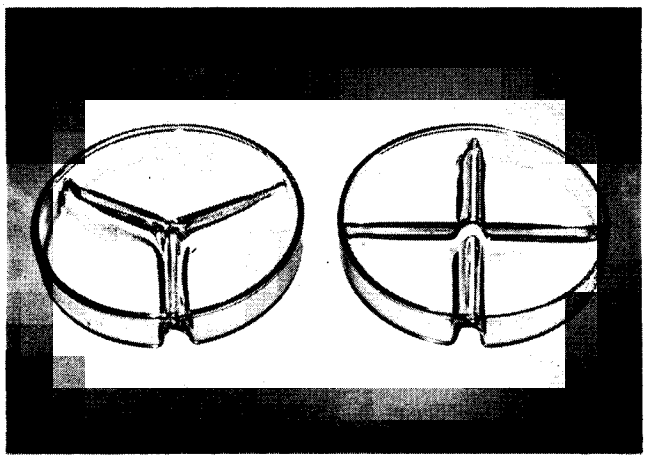


FIG. 1. Petri dish bottoms with 3 and 4 divisions used in comparison of film formation on brines of different salt concentration. These dishes have also been used for cultivation of giant colonies of yeasts on different solid media. The bottoms were made from regular pyrex dishes having a depth of 15 mm., a diam. of 90 mm., and take 100 mm. diam. cover. The divisions are approximately 8 to 10 mm. in height and were made by softening the bottoms with a flame and forming the divisions seen above.

the latter three genera have not, to our knowledge, been reported before in connection with film formation on salt-stock vegetable brines. Furthermore, yeasts belonging to the genus *Zygosaccharomyces* heretofore have not been considered associated with yeast genera capable of rapid and luxuriant film formation. This characteristic could easily be overlooked, because the *Zygosaccharomyces* from brine produce only a thin scum on liquid media containing certain of the sugars used in the fermentation tests. Also, no film is produced in alcohol in contrast to most yeasts classed as film-forming types. However, luxuriant films are rapidly produced in liquid media containing 5 to 20 percent salt.

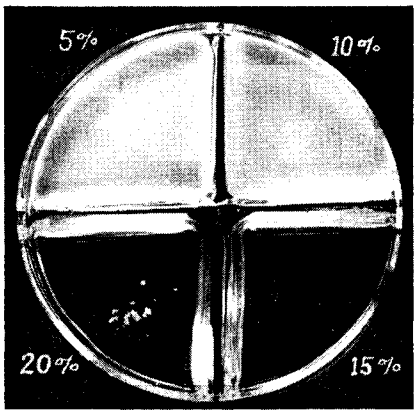
Distribution of Genera

Information on the distribution of the film yeasts, according to the five representative geographical brining areas, is shown in Table 2. Yeasts belonging to the genus *Debaryomyces* were most widespread. They were found in all five states and were also the most frequently isolated from the commercial brine films. The *Zygosaccharomyces* were found in all northern states.

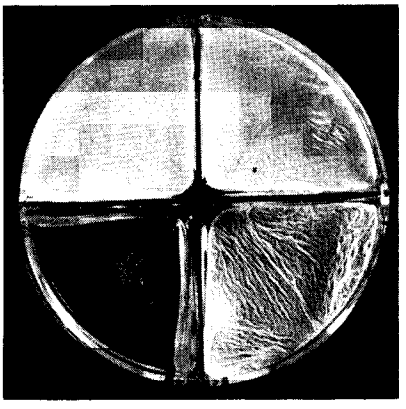
The *Endomycopsis* and *Candida* were limited to brining areas in North Carolina. The *Endomycopsis* genus was isolated from brines with a rather wide range of salt concentration (Table 1) and it seems reasonable that this was not a deciding factor for limiting their distribution. The *Candida* came from the films of two vats of genuine dills having brines of about 5 percent strength. This points to a direct relationship between brine strength and the possibility of finding isolates of this species of *Candida*.

Salt Tolerance

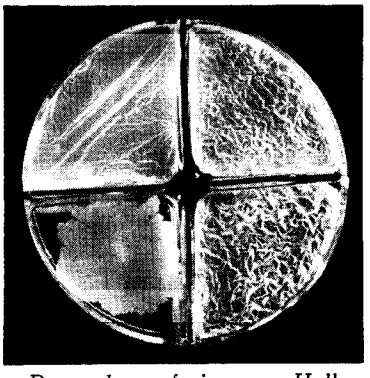
Representative cultures of all genera were tested for salt tolerance, as indicated by film formation on cucumber brine (see Figure 2). The rate of film formation was considered important, since this probably would be



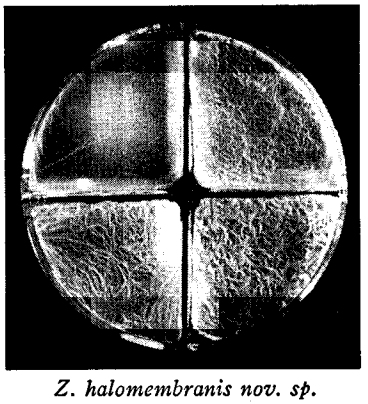
C. krusei



E. ohmeri nov. sp.



D. membranaefaciens var. *Holl.*



Z. halomembranis nov. sp.

FIG. 2. Growth of film yeast isolates on cucumber brines containing 5, 10, 15, and 20 percent salt by weight; 1-week's incubation at 25° C. After 2-week's incubation, the *Debaryomyces* species formed a heavy wrinkled film on 20 percent salt and the *Endomycopsis* formed a slight film; the *Candida* did not grow above 10 percent salt.

an influencing factor as to the predominance of a given yeast on a vat brine under suitable conditions of growth. All genera except the *Candida* isolates were capable of rather high salt tolerance as indicated by range of brine strengths from which they were obtained (Table 1).

No outstanding differences in final tolerance were found between the isolates of *Debaryomyces* and *Zygosaccharomyces*. Both groups produced luxuriant, folded films on cucumber brines at 20 percent salt by weight; isolates of the former genus took about 10 days, whereas the latter required only 5 days. The rate of film formation at lower concentrations (5, 10, and 15 percent) was essentially the same for both genera. The high salt tolerance of the *Debaryomyces* has been demonstrated earlier *(7)* and because of this these yeasts were considered to be more widely distributed on salt brines. We are in general agreement with this; however, another important factor is their ability to assimilate a wide variety of carbon compounds as a source of energy.

The cultures of *Endomycopsis* produced heavy, folded films at 15 percent salt in 5 to 7 days but there was little evidence of film formation at 20 percent until about 2 weeks. After that a rather thin film appeared. The isolates of this genus were divided into two groups: Those from laboratory source and those from commercial source. Cultures from both groups showed no difference with respect to salt tolerance, although certain differences in cultural characteristics and film formation on ethyl alcohol indicated a difference as to variety.

The *Candida* cultures showed a very clear-cut response to increasing salt concentrations. Film formation was absent on 15 and 20 percent brines. Ten percent appeared to be about the maximum, and the film at this strength was thinner than at 5 percent. This characteristic probably accounts for not finding more isolates of this yeast, since only 6 brines were sampled in the 5 to 10 percent range. The results for 3 *Pichia* cultures which were isolated from low-salt, high-acid content pickle products were essentially the same as for the *Candida* cultures. Mrak and Bonar *(7)* reported that their cultures of *Pichia* and *Mycoderma* isolated from food brines were "barely able to grow in brines containing 15.1 percent salt."

In the present work no cultures of *Mycoderma* were obtained. In the industry this genus is considered to be responsible for the films on salt-stock brines. But this investigation, as well as the work by Mrak and Bonar *(7)*, would indicate that species of *Mycoderma* would not be expected to predominate on brines in commercial practice (for brining cucumbers) where the starting strength is usually 7 to 10 percent by weight and is raised to 15 to 17 percent in about 6 weeks. Species of genera that are very salt-tolerant and fast-growing on brines of this strength would no doubt monopolize the surface of the brine and would be expected to restrict the less salt-tolerant types such as *Mycoderma* and *Pichia*. Earlier reports on film yeasts on brines were made prior to more modern taxonomic methods for yeast study *(1, 2, 10)* For example, without a test for mycelium production, differentiation between certain species of *Candida* and *Mycoderma* would be rather difficult.

Cultural and Biochemical Properties and Classification

Debaryomyces: Eighteen of the 22 cultures placed in this genus formed a uniform group according to the usual taxonomic tests. They are very poorly fermentative; glucose and sucrose may be weakly fermented. All the 7 test sugars are assimilated with a white, climbing film. On ethyl alcohol a dull, white film is formed with a fine network of folds. The film remains intact for a long period. On wort a moderately heavy film, light tan in color, with a network of short fine folds is produced. Potassium nitrate is not utilized. Cells, occurring in clusters, are round to slight oval and average 4-6 microns in diameter. Sporulation is after either isogamic or heterogamic copulation with 1 spore per ascus. Spores are spherical, rough, 3-4 microns in diameter with centrally located oil drop. Asci are 8 to 10 microns in length. Pseudomycelium is absent on corn meal agar. Glucose agar slant growth is chalky white, raised, folded. Folds change from white to tan, and then to brown color on vegetable juice agar as sporulation takes place. Salt-tolerance: heavy film formation at 20 percent; maximum not determined, probably close to saturation (26.4%). Type strains are FY-21, FY-22, FY-36; NFY-20.

Four cultures differ from the above descriptions in that they are inclined to be smooth on slants and produce thin films on ethyl alcohol. More precise and extensive carbon assimilation tests, such as reported by Wickerham and Burton *(11)* for the *Hansenula,* will be required to make any distinct separation. The brine isolates of *Debaryomyces* were not placed as to species other than on a tentative basis and are similar to several species that have been described *(10)*. But a number of the descriptions are so similar that separation is extremely difficult. The isolates of the present work are similar to *Debaryomyces membranaefaciens* var. *Hollandicus* Lodder.[h]

Endomycopsis: The isolates placed in this genus consisted of 12 cultures obtained from films on commercial brines, and 14 from films on cucumbers brined under laboratory conditions. All were placed in a new species of *Endomycopsis* based chiefly on the sugars fermented, and on the ability to produce a film on ethyl alcohol. The latter characteristic is negative for the species previously listed *(10)*. The name *Endomycopsis ohmeri* is suggested for the group and they are named for the late Harvey B. Ohmer who assisted in collecting the first isolates from films in eastern North Carolina. The type species represents the 12 isolates from commercial sources and are characterized by a heavy wrinkled film on ethyl alcohol. Varietal rank *(E. ohmeri* var. *minor)* was given to the 14 isolates from laboratory source and these cultures are readily differentiated by a membrane-type of film on ethyl alcohol and difference in the type of slant growth.

The characteristics for the new species and variety are

[h] Further separation is planned later in cooperation with Dr. L. J. Wickerham and coworkers of the Northern Regional Research Laboratory (Peoria), when they have completed the carbon assimilation patterns for the species of the *Debaryomyces* genus.

as follows: *Endomycopsis ohmeri nov. sp.:* Glucose, sucrose, galactose, and raffinose (⅓) are fermented. Maltose, lactose, and melibiose are not fermented but maltose and lactose are assimilated with formation of a smooth film. On ethyl alcohol, a white, heavy, wrinkled film is formed with close folds; broth is clear and the underneath portion of film is ragged; film wets and disintegrates in about 10 days. On wort, heavily wrinkled film is formed with a network of short folds, dark tan in color; growth ragged underneath film. Nitrate (KNO_3) is not utilized. The cells are slender, oval, very elongated; 3 x 4 microns to chains of 2 x 15 to 30 microns and branched. Hat-shaped spores produced on vegetable juice agar 1 to 4 per ascus; sporulation scant. Pseudomycelium abundant on cornmeal agar; evidence of true mycelium found. Glucose agar slant growth chalky white, coarsely netted, wet underneath surface. Salt tolerance: heavy film formation at 15 percent; scant to weak at 20 percent in 3 weeks. Type strains, FY-15, FY-25 and Y-470.

Endomycopsis ohmeri sp. nov.: Fermentationem glucosii, sucrosii, galactosii et raffinosii (⅓) inducens, sed non maltosii, lactosii, nec melibiosii; maltosium lactosiumque assimilantur pellicula levi; pellicula in alcoholi ethylico alba, crassa, plicis densis rugosa, infra lacterata liquido claro, humida, in circa 10 diebus disrumpens; in musto dense rugosa, fusca, plicis brevibus reticulata, infra lacertata; nitratum non utitur; cellulae angustae, elongato-ovales, 3-4 μ vel in catenis ramosis 2 x 15-30 μ longis; ascosporae pileiformes in agaro succus vegetabilium, 1-4 in quoque asco, sporulatione parca; pseudomycelium in agaro farinae-zeae abundans; mycelium verum evidens; auctus in agaro glucoso cretaceo-albus, crasse reticulatus, infra madidus; toleratio salis; formatio gravis pelliculae ad 15%, parca vel debilis ad 20% in 3 hebdomadibus.

Germina typica FY-15, FY-25, et Y-470.

Endomycopsis ohmeri var. *minor nov. var.:* Glucose, sucrose, galactose and raffinose (⅓) are fermented. Maltose, lactose and melibiose are not fermented but maltose is assimilated with a very heavy growth. On ethyl alcohol, a white, smooth intact membrane-type of film is formed which falls in a few days, either as a whole or in sections. On wort a heavy, wrinkled film with long folds is formed which is tan to brown in color. Nitrate (KNO_3) is not utilized. The cells are large oval to cylindrical in shape and may be in long chains. Cells 4 x 7-20 microns. Hat-shaped spores produced on vegetable-juice agar, 1 to 4 per ascus; sporulation scant. Pseudomycelium abundant on corn-meal agar; evidence of true mycelium found. Glucose agar slant growth yellowish, dry, mealy, finely netted. Salt-tolerance: Heavy film formation at 15 percent; scant to weak at 20 percent in 3 weeks. Type strains, FY-1, FY-7, and FY-14.

Endomycopsis ohmeri var. *minor* var. nov.: Fermentationem glucosii, sucrosii, galactosii et raffinosii (⅓) inducens, sed non maltosii, lactosii nec melibiosii; maltosium assimilatur aucto gravidissimo; pellicula in alcoholi ethylico alba, levis, integra, membranacea, in diebus paucas vel intacta vel in fragmentis delabens, in musto gravida, plicis longis rugosa, alutacea vel brunnea; nitratum non utitur; cellulae magnae ovales usque cylindricae, interdum in catenis longis, 4 x 7-20 μ; ascosporae pileiformes in agaro succus vegetabilium, 1-4 in quoque asco, sporulatione parca; pseudomycelium in agaro farinaezeae abundans; mycelium verum evidens; auctus in agaro glucoso flavidulus, aridus, farinosus, tenuiter reticulatus; tolerantia salis: formatio gravida pelliculae ad 15%, parca vel debilis ad 20% in 3 hebdomadibus.

Germina typica FY-1, FY-7 et FY-14.

Saccharomyces, subgenus *Zygosaccharomyces:* The 9 isolates placed in this genus were a uniform group and in view of their unusual characteristic of rapid and luxuriant film formation on high salt-content brines, they were considered to belong to a new species. The name *Zygosaccharomyces halomembranis* is suggested to conform with the characteristic that is so distinctive.

The complete description is as follows: *Zygosaccharomyces halomembranis:* Glucose and maltose are fermented; action on sucrose is variable, but this sugar may be fermented weakly. Lactose, galactose, raffinose, and melibiose are not fermented. Sucrose, galactose, and raffinose assimilated with heavy ring and sediment. Good growth in ethyl alcohol; ring but no film formation. Nitrate (KNO_3) not utilized. Pseudomycelium not found on corn-meal agar. Cells generally round to slight oval, 5 to 7 microns in diameter, occurring in clusters. Sporulation after either isogamic or heterogamic copulation 1 to 4 spores per ascus, usually 1 or 2. Spores average 3.5 x 4 microns in size, usually oval shape. Glucose slant growth, light cream color, raised, dull and may be finely papillate in center area of growth. Salt tolerance: Heavy, rapid film formation at 20 percent; maximum not determined, probably close to saturation (26.4%). Film formation negligible to absent in liquid media without salt. Type strains NFY-6, NFY-26.

Zygosaccharomyces halomembranis sp. nov.: Fermentationem glucosii, maltosii que etiam interdum sucrosii leviter inducens, sed non lactosii, galactosii, raffinosii nec melibiosii; sucrosium galactosium et raffinosium assimilantur, annulo et crassimine gravidis; auctus in alcoholi ethylico bonus, annulo praesenti sed pellicula carenti; nitratum non utitur; pseudomycelium non visum in agaro farinaezeae; cellulae plerumque rotundae vel subovales, 5-7 μ in diam., in greges confertae; sporulatio post copulationem isogamicam vel heterogamicam; ascosporae 1-4 (generaliter 1-2) in quoque asco, 3.5-4 μ, plerumque ovales; auctus in medio glucoso pallide cremeus, elevatus, obscurus, centro subtiliter papillatus; tolerantio salis: formatio pelliculae gravida et rapida ad 20%, maxima indeterminata, fortasse 26.4%, in mediis liquidis sine sale tenuis vel absens.

Germina typica NFY-6, NFY-26.

These characteristics are essentially the same as those for the 9 glucose, maltose fermenting cultures of *Zygosaccharomyces* recently described by Etchells and Bell *(3),* but which were not placed as to species. These

yeasts came from subsurface brine samples of a sheltered vat which had a surface film. However, the ability to form films on brines was missed because salt-tolerance tests using liquid media were not run.

A test was made on the ability of three strains of *Z. halomembranis nov. sp.* to form films on cucumber brines of different salt concentration as compared with species and varieties in the genus *Zygosaccharomyces* which have similar carbon assimilation reactions. The 16 yeasts, selected and supplied by Dr. L. J. Wickerham of the Northern Regional Research Laboratory (NRRL) were: 1. *Z. mellis,* 2. *Z. barkeri,* 3. *Z. japonicus,* 4. *Z. japonicus* var. *soya,* 5. *Z. major,* 6. *Z. nadsonii,* 7. *Z. richteri,* 8. *Z. dairensis,* 9. *Z. rugosus* (2 strains), 10. *Z. amoeboideus,* 11. *Z. cavarae* var. *beauverie,* 12. *Z. felsineus,* 13. *Z. gracilis,* 14. *Z. major* var. *threntensis,* 15. *Z. salsus* var. *saccharosum,* and 16. *Z. polymorphus.* The above NRRL cultures were inoculated by loop from the original tubes into cucumber brines containing 5, 10, 15 and 20 percent salt by weight. Prior to inoculation into the test brines, the 3 strains of *Z. halomembranis nov. sp.* had not been on salt-containing media for approximately 2 years.

After 5 days the 3 strains of *Z. halomembranis* formed heavy folded films on the 15 and 20 percent salt brines and moderately heavy films on the 5 and 10 percent brines, whereas no film formation was observed for any of the 16 NRRL cultures. After 2-, 4-, and 8-week incubation periods the results were the same with respect to film formation for the 16 species tested. However, after two weeks heavy ring growth, turbidity, and sediment was noted at 10 percent salt for one species, *Z. mellis.* Furthermore, after 4 week's incubation all the 17 cultures tested had slight to moderate ring growth and increased sediment in 10 percent brine, and the first 8 cultures listed above showed definite signs of ring growth at 15 percent, but not at 20 percent.

The results indicate that the 16 species of *Zygosaccharomyces,* after 8 weeks' incubation, were not capable of film formation on cucumber brines ranging in strength from 5 to 20 percent salt by weight although a number of species were able to grow reasonably well in brines containing up to 15 percent salt. Thus *Z. halomembranis* can be readily separated from other *Zygosaccharomyces* species and varieties having similar fermentation and carbon assimilation reactions by virtue of rapid and luxuriant film formation on high salt-content brines or salt-containing liquid media.

Candida: The 4 cultures in this genus were placed as *C. krusei* (A. Cast.) Berkhout and are characterized by the fermentation of glucose only. Sucrose, maltose, galactose, lactose, raffinose and melibiose are not fermented nor assimilated. A very thin film appears on these sugars but it is attributed to assimilation of the slight amount of acid in the basal medium. On ethyl alcohol, a white, moderately heavy film, composed of a fine network of folds; on wort, a similar film is formed but it is light tan in color. Potassium nitrate is not utilized. Cells are large, rod-like, cylindrical in shape; also large oval cells. Cells average 2.5-3 x 4 to 35 microns and occur in chains. Pseudomycelium is abundant on corn-meal agar; septated mycelium is present. No spores are observed on several sporulation media, observed over several months. Glucose slant growth is white, dull, flat, not folded, or wrinkled; edge spreading and fuzzy. On vegetable juice agar a very fine network develops in the center of slant growth. In respect to salt tolerance; moderate film formation at 10 percent but none at 15 percent. Type strains are FY-18, FY-19, FY-20.

De-esterification of pectin: An interesting biochemical property was observed in connection with the growth of cultures from two of the film yeast genera in a liquid medium at pH 5 containing 0.5 percent salt, 0.5 percent peptone, 0.25 percent yeast extract, and 0.5 percent citrus pectin (purified, free of sugars). Representative isolates of *Endomycopsis* and *Debaryomyces* were capable of de-esterifying the pectin in the medium so that a gel was formed upon the addition of one drop of a 20 percent calcium chloride solution to 0.5 ml. of the culture medium. At present, the exact nature of the reaction has not been determined. The *Zygosaccharomyces* and *Candida* isolates obtained in the present study were negative for the above test; however, three cultures of *Candida,* representing two different species, previously isolated from brines were positive.

Summary and Conclusions

A taxonomic study of 47 yeast cultures associated with the production of surface films on 40 commercial cucumber pickle brines ranging in salt content from 5-19 percent salt, and located in five states, is presented.

The cultures were classified as follows: *Debaryomyces membranaefaciens* var. *Hollandicus* Lodder 18 isolates; *Endomycopsis ohmeri nov. sp.,* 12; *Zygosaccharomyces halomembranis nov. sp.,* 9; and *Candida krusei* (A. Cast.) Berkhout, 4. Four cultures put in the *Debaryomyces* genus were not placed as to species.

An additional 14 isolates of *Endomycopsis* obtained from a like number of films on cucumbers brined under laboratory conditions were given varietal rank in the new species as *Endomycopsis ohmeri* var. *minor.*

Yeasts belonging to the genus *Debaryomyces* were the most widespread, and were found on brines in all five states. Their high salt tolerance coupled with the ability to assimilate a large number of compounds as a source of carbon were considered to be important factors in their frequency of occurrence and distribution. Yeasts belonging to the genera *Zygosaccharomyces* and *Endomycopsis* have not previously been considered associated with film formation on cucumber brines or other types of food brines.

Sixteen species and varieties of *Zygosaccharomyces* with carbon assimilation reactions similar to the new species *Z. halomembranis* were tested for film formation on cucumber brines at 5, 10, 15 and 20 percent salt concentration. Negative results for film formation were obtained for the known cultures observed for a period up to two months, whereas heavy films were produced by *Z. halomembranis* in five days.

Representative cultures placed as species in the *Debaryomyces* and *Endomycopsis* genera were capable of de-esterifying citrus pectin in a cultural medium.

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