

# Pectin Hydrolysis by Certain Salt-Tolerant Yeasts<sup>1</sup>

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During the brine fermentation of cucumbers for pickles, Bell *et al.* (1950) have reported finding a fungal-type polygalacturonase-like enzyme which splits the polymer linkage of pectic substances. They definitely established this enzyme with the softening type of spoilage of salt-stock pickles brined under commercial conditions. With the identity of the softening agent known, an inquiry into its origin is of interest. The principal possibilities as to source are: the microbiological activity occurring during the curing and subsequent storage in brine, and the nature of the green cucumber fruit that is brined. The present work considers one phase of the first category, namely, the possible relationship of brine yeasts to softening as indicated by their ability to hydrolyze citrus pectin in cultural media. The method used in this study was devised as a rapid screening technique for such biochemical changes of pectin as indicated by a calcium pectate gel or the loss of such gel properties. The changes in pectin are not taken to be specific enzyme measurements for polygalacturonase and pectinesterase; nevertheless, such a screening technique is most valuable for qualitatively identifying yeasts as potential sources of pectic enzymes.

Pittman and Cruess (1929) reported on the hydrolysis of a 1 per cent apple pectin by various microorganisms as indicated by change in viscosity, decrease in pectin content, and loss of gelling power of the pectin. The yeast cultures tested—*Saccharomyces cerevisiae*, *Saccharomyces ellipsoideus*, and *Mycoderma* species—were reported to have no noticeable effect on the pectin.

Luh and Phaff (1951) found 6 cultures of yeasts (*Saccharomyces fragilis*, *S. fragilis* var. no. 351, *Saccharomyces thermantitonus*, *Torulopsis kefyi*, *Candida pseudotropicalis* var. *lactosa*, and *Candida pseudotropicalis*) that were capable of causing a noticeable change in pectin broth containing minerals and glucose. Clarification of the opaque pectin solution to a water-clear liquid with the formation of a sediment was the criterion used to determine hydrolytic action. According to Luh and Phaff, these 6 cultures can all be identified with *S. fragilis* and a variety of this species, along with its

imperfect form *Candida pseudotropicalis* and certain varieties of the latter. *S. fragilis* var. no. 351 caused the most rapid clarification of the citrus pectin medium, so they further studied and reported its enzyme properties (Luh and Phaff, 1951, 1954a, 1954b). They named the enzyme yeast polygalacturonase because they found it to be different from that of mold origin in that the pectic acid substrate was only partially hydrolyzed. Demain and Phaff (1954) further characterized yeast polygalacturonase and reported that ultimately galacturonic and digalacturonic acids were the end products of pectic acid hydrolysis. Roelofsen (1953) referred to his little-known earlier studies (published in 1936 in the Dutch language) which reported the ability of certain yeasts to attack cell-wall pectin. These yeasts belonged to the genera *Candida*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces*, and many of the cultures were isolated from fermenting cocoa beans from Java and West Africa. Included in this study were species of *S. fragilis* and its imperfect form *C. pseudotropicalis*. Roelofsen (1953) concluded that certain species of yeasts do contain true polygalacturonase and not merely a depolymerase.

Matus (1948), in connection with a study on the activity of pectinase (*syn.* polygalacturonase) reviewed the reported sources of this enzyme. Numerous genera and species of microorganisms are listed as a source of pectinase, but none that would be classified as yeasts was reported. Other reviews on the subject of pectin and pectic enzymes (Joslyn and Phaff, 1947; Phaff and Joslyn, 1947; Lineweaver and Jansen, 1951) gave no additional information on the hydrolysis of pectin by yeasts. Further, there appears to be no record of pectinesterase, an enzyme which catalyzes the hydrolysis of the ester bonds of pectic substances, as being produced by yeasts, although Etchells and Bell (1950a), in connection with a taxonomic study of film yeasts from cucumber brines, mentioned obtaining de-esterification of citrus pectin by species of yeasts belonging to the genera *Endomycopsis* and *Debaryomyces*.

## MATERIALS

*Origin of cultures.* Yeasts associated with the fermentation of brined cucumbers are divided into two general groups: those which produce a gaseous fermentation below the surface of the brines, and those which produce luxuriant films on the brines exposed to air but

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sheltered from direct sunlight. Species belonging to the genera *Torulopsis*, *Brettanomyces*, *Hansenula*, *Zygosaccharomyces*, and *Torulospira* have been shown (Etchells and Bell, 1950b; Etchells *et al.*, 1952) to be the principal types present during the fermentation under conditions typical of the southern and northern brining areas of the United States. Film formation on commercial cucumber brines has been attributed to species of *Debaryomyces*, *Endomycopsis*, *Zygosaccharomyces*, and *Candida* (Etchells and Bell, 1950a). Most of the species that belong to both of the general groups mentioned have a high tolerance to salt and acid. These are two important qualities, in addition to the production of a polygalacturonase-like enzyme, that would be required of any group of microorganisms associated with the softening of brined cucumbers.

In addition to the two groups just named, yeast isolates from related brined, salted, and pickled products<sup>3</sup> were investigated as well as known species from several culture collections. A few cultures from sources other than brined or pickled material were tested because their history indicated the possibility of pectolytic activity. Of these, 4 cultures of *S. cerevisiae* from spoiled orange juice concentrate were obtained from Dr. H. H. Hall of the Southern Utilization Research Branch, U. S. Department of Agriculture, New Orleans, Louisiana, and were considered by Hall and Teunisson (personal communication, 1948) to have pectolytic properties. Four other cultures with a history of pectolytic activity (Luh and Phaff, 1951) were received from Dr. H. J. Phaff, Food Technology Department, University of California, Davis, California. These were *S. fragilis*, *S. fragilis* var. no. 351, *Candida pseudotropicalis*, and *C. pseudotropicalis* var. *lactosa*.

#### METHODS

**Culture media.** The cultures were grown in basal liquid medium consisting of 0.5 per cent citrus pectin,<sup>4</sup> 0.5 per cent peptone, 0.5 per cent salt, and 0.25 per cent yeast extract. The medium was modified as follows as to pH and glucose content; medium I, adjusted to pH 5.0 without glucose; medium II, adjusted to pH 5.0 plus 1.0 per cent glucose; medium III, adjusted to pH 5.0 plus 0.2 per cent glucose; medium IV, adjusted to pH 3.5 plus 0.2 per cent glucose. The media were put

<sup>3</sup> Namely, fermenting sweet pickle; brined and dry-salted green beans; brined bacon; films on processed dill pickles in glass; sour pickles; fermenting pasteurized sweet pickle; film on sauerkraut; brined, unshelled green peas; brined sweet pepper hulls; and corn-apple silage. Yeast cultures from these sources are from the authors' collection and are designated by the letters "FFL."

<sup>4</sup> Obtained from the California Fruit Growers Exchange, Ontario, California, under the name Pectinum NF VIII. It is probable that other products of essentially the same composition would prove satisfactory.

in culture tubes in approximately 15-ml amounts and sterilized in racks at 10 lb of pressure for 15 min and then promptly cooled in water. This procedure was followed to minimize the effect of heat on the pectin in the media. The yeasts were inoculated by loop into the test media from young (1-week-old) vegetable juice agar (Etchells and Bell, 1950b) slant cultures. Determinations for pH were made by the glass electrode; pH adjustment of the pectin media was done with 5 per cent tartaric acid. All cultures were tested for pectin hydrolysis, after 30 days of incubation at 30 C, as described below.

**De-esterification of pectin (DE).** This test was carried out in small test tubes (8 by 75 mm) and consisted of adding 1 drop of 20 per cent calcium chloride solution to a 0.5-ml sample of the culture medium. The formation of a calcium pectate gel (as compared to none in the uninoculated control) demonstrated de-esterification of the pectin resulting from yeast growth. This test does not indicate the mode of action of the de-esterification, such as by pectinesterase, alkali, or acid (Joseph *et al.*, 1949; Baker, 1942).

**Glycosidic hydrolysis of pectin (GH).** If no gel occurred in the above test with cultures that had shown definite growth, it could mean that the yeast was either negative for de-esterification of pectin, or the hydrolysis had proceeded further than pectic acid. To determine which condition existed in the non-gelled tubes, the pectin in the 0.5 ml of culture medium was saponified by the addition of 1 drop of 1 N sodium hydroxide. After 30 min, if no gel then occurred, it was considered a positive test for glycosidic hydrolysis. If a gel occurred, the culture was considered negative for both de-esterification and glycosidic hydrolysis.

Yeasts that showed a positive reaction for glycosidic hydrolysis in this screening procedure were tested further to determine if a polygalacturonase-like enzyme had been secreted into the culture medium. This was done by measuring the change in viscosity of a pectin solution caused by a sample of the culture medium. This technique has been adequately described by Bell *et al.*, (1950).

#### RESULTS

A summary of the results on the ability of the 139 yeasts tested to hydrolyze pectin follows. The reactions listed for de-esterification and glycosidic hydrolysis in most cases are based on the combined observations from tests made using the 4 cultural media. Inasmuch as both screening tests are only considered to be qualitative in nature, the degree of activity is not indicated. The yeasts are listed according to genus and species followed by the type culture designation,<sup>5</sup> number of

<sup>5</sup> Type culture abbreviations and location of culture collections: The first four species of the genus *Zygosaccharomyces* were obtained from Dr. A. G. Lochhead, Dominion of Canada

strains tested, and the ability to hydrolyze pectin as indicated by the presence of the letters DE for de-esterification, or GH for glycosidic hydrolysis.

*Zygosaccharomyces*: *Z. richteri* (M-1) 1; *Z. rugosus* (139) 1; *Z. nectarophilus* (N-4) 1; *Z. nussbaumeri* (J-7) 1; *Z. halomembranis* n. sp. (FFL-1000) 7; *Z. globiformis* (FFL-481) 11; *Z. species A* (FFL-245) 1; *Z. species B* (FFL-484) 5.

*Debaryomyces*: *D. matruchoti* (NRRL-833) 1, DE; *D. membranaefaciens* var. *Hollandicus* (FFL-95) 9, DE; *D. species smooth* (FFL-94) 5, DE; *D. species A* (FFL-109) 1; *D. species B* (FFL-121) 1, DE; *D. species C* (FFL-128) 1, DE; *D. globosus* (FFL-130) 1, DE; *D. species E* (FFL-13 BA) 1, DE.

*Hansenula*: *H. saturnus* (NRRL-12) 1, DE; *H. subpelliculosa* (NRRL-1009, FFL-209) 13; variable for DE; *H. anomala* (NRRL-778) 4, DE.

*Brettanomyces*: *B. lambicus* (NRRL-1413) 1; *B. bruxellensis* (NRRL-1411) 1; *B. anomalus* (NRRL-1415) 1; *B. claussenii* (NRRL-1414) 1; *B. bruxellensis* var. *non-membranaefaciens* (NRRL-1412) 1; *B. versatilis* (FFL-35) 6; *B. versatilis* var. (FFL-38) 2; *B. sphaericus* (FFL-44) 2.

*Torulopsis*: *T. bacillaris* (NRRL-1444) 1; *T. stellata* (NRRL-1446) 1; *T. lactis-condensi* (CBS) 1; *T. utilis* (DOW-33) 1; *T. caroliniana* (FFL-304) 4; *T. holmii* (FFL-312) 2.

*Saccharomyces*: *S. fragilis* (UC-106) 1 GH; *S. fragilis* var. (UC-351) 1 GH; *S. cerevisiae* (SRRL-D6) 4 GH; *S. globosus* (FFL-273) 4; *S. species* (FFL-287) 1; *S. ellipsoideus* (MSC) 1, weak GH; *S. species* (SB-139) 1.

*Candida*: *C. krusei* (FFL-59) 3, DE; *C. tropicalis* (DUH) 1, DE; *C. pseudotropicalis* (UC-143) 1, weak GH; *C. pseudotropicalis* var. *lactosa* (UC-39), 1; *C. pulcherrima* (NRRL-412) 1, DE; *C. krusoides* (FFL-55) 3, DE; *C. species A* (FFL-58) 1, DE.

*Torulasporea*: *T. rosei* (FFL-380) 6.

*Endomycopsis*: *E. ohmeri* (FFL-149) 3, DE; *E. ohmeri* var. *minor* (FFL-152) 2, DE.

*Pichia*: *P. alcoholophila* (NRRL-368) 3; *P. membranaefaciens* (NRRL-847) 1.

*Kloeckera*: *K. africana* (NRRL-1274) 1; *K. japonica* (NRRL-1382) 1; *K. magna* (FFL-237) 1.

*Mycoderma*: *M. lafarrii* (NRRL-936) 1; *M. cerevisiae* (NRRL-99) 1.

*Rhodotorula*: *R. species A* (FFL-251) 1, DE; *R. species B* (FFL-254) 1, DE.

*Zyghansenula*: *Z. californica* (NRRL-1425) 1, DE (weak).

*Zyggopichia*: *Z. farinosa* (NRRL-118) 1, DE.

Of the species tested in the 15 genera, those in 7, namely, *Zygosaccharomyces*, *Brettanomyces*, *Torulopsis*, *Torulasporea*, *Pichia*, *Kloeckera*, and *Mycoderma*, were considered negative for pectin hydrolysis under the conditions employed. These yeasts grew poorly, if at all, in the pectin medium without glucose (medium I). Most of the species tested of *Debaryomyces*, *Hansenula*, *Candida*, *Endomycopsis*, *Rhodotorula*, and *Zyggopichia* were able to de-esterify pectin but were negative for glycosidic hydrolysis. De-esterification by *Hansenula subpelliculosa* was variable; 8 were positive, 3 were negative, and 2 were weak to doubtful. A clear-cut de-esterification reaction was not obtained for the single species of *Zyghansenula* (*Z. californica*) tested, or for one of the species of *Saccharomyces* (*S. ellipsoideus*). Three other species of *Saccharomyces* also gave a negative test for de-esterification, but since they were found positive for glycosidic hydrolysis, the former test is inconclusive.

Six cultures, representing 2 species and a variety are listed as being capable of glycosidic hydrolysis of pectin (*S. fragilis*, *S. fragilis* var., and *S. cerevisiae*). Further, the culture media from these yeasts, when added to a 3 per cent pectin solution buffered at pH 4, caused a marked loss in viscosity over that of heat-inactivated controls. It would appear that the glycosidic hydrolysis in these cases was due to a polygalacturonase-like enzyme as previously demonstrated by Luh and Phaff (1951, 1954a, 1954b) and Roelofsen (1953). Two cultures, *S. ellipsoideus* and *C. pseudotropicalis*, were weak to doubtful for glycosidic hydrolysis. Further studies by the authors (unpublished data, 1956) have demonstrated the ability of *S. fragilis* (UC-106) and *S. fragilis* var. (UC-351) to produce complete softening of cucumbers in 1-quart jars of pasteurized material equalized at 4 to 5 per cent salt, 0.25 per cent lactic acid and pH 4.0. In contrast, representative species of yeasts responsible for the gaseous fermentation of commercially brined cucumbers (that is, spp. of *Torulopsis*, *Brettanomyces*, *Hansenula*, *Zygosaccharomyces*, and *Torulasporea*) gave no evidence of softening action.

The influence of the glucose content and pH adjustment of the culture medium upon de-esterification of pectin is presented in table 1. Only representative cultures of species that were found to be capable of hydrolysis are listed. Pectin medium III (pH 5.0 and 0.2 per cent glucose) was found to give the largest number of positive de-esterification reactions for species in the genera *Debaryomyces*, *Hansenula*, *Candida*, *Endomycopsis*, *Rhodotorula*, *Zyghansenula*, and *Zyggopichia*. Only

Department of Agriculture; FFL = Food Fermentation Laboratory (Authors' Collection), Raleigh, North Carolina; NRRL = Northern Utilization Research Branch (U.S.D.A.), Peoria, Illinois; CBS = Centraalbureau voor Schimmelcultures, Delft, Holland; DOW = Dow Chemical Co., Midland, Michigan; UC = University of California, Davis, California; DUH = Duke University Hospital, Durham, North Carolina; MSC = Michigan State University, East Lansing, Michigan; SRRL = Southern Utilization Research Branch (U.S.D.A.), New Orleans, Louisiana; SB = Standard Brands Research Laboratory, Hoboken, New Jersey.

TABLE 1. Influence of 4 cultural media on the ability of yeasts to de-esterify 0.5 per cent pectin

Yeast	Medium I (No Glucose, pH 5.0)		Medium II (1% Glucose, pH 5.0)		Medium III (0.2% Glucose, pH 5.0)		Medium IV (0.2% Glucose, pH 3.5)	
	Growth*	DE†	Growth	DE	Growth	DE	Growth	DE
<i>Debaryomyces</i>								
<i>D. matruchoi</i> .....	H	+	H	-	H	+	S	+
<i>D. membranacefaciens</i> var. <i>Hollandicus</i> ....	H	+	H	-	H	+	S	-
<i>D. species B.</i> .....	H	+	H	-	H	+	M	-
<i>D. species C.</i> .....	M	+	H	-	H	+	M	-
<i>D. globosus</i> .....	M	+	H	-	M	+	M	-
<i>D. species (smooth)</i> ...	H	+	H	-	M	+	H	-
<i>D. species E.</i> .....	M	+	H	-	M	-	M	-
<i>Hansenula</i>								
<i>H. saturnus</i> .....	M	+	H	-	H	+	H	-
<i>H. anomala</i> .....	H	+	H	+	H	+	H	-
<i>H. subpelliculosa</i> (NRRL-1009).....	M	-	M	-	M	+	M	-
<i>H. subpelliculosa</i> (FFL-209).....	S	-	M	-	M	+	M	-
<i>Candida</i>								
<i>C. krusei</i> .....	H	-	H	-	H	+	H	-
<i>C. tropicalis</i> .....	H	+	H	+	H	+	H	-
<i>C. krusoides</i> .....	H	+	H	-	M	+	S	-
<i>C. pulcherrima</i> .....	M	+	H	-	H	+	M	-
<i>C. species A.</i> .....	M	+	H	-	M	+	S	-
<i>Endomycopsis</i>								
<i>E. ohmeri</i> .....	H	+	H	±	H	+	H	-
<i>E. ohmeri</i> var. <i>minor</i> ..	H	+	H	-	H	+	H	-
<i>Rhodotorula</i>								
<i>R. species B.</i> .....	M	+	H	-	M	+	M	-
<i>Zygothansenua</i>								
<i>Z. californica</i> .....	M	-	H	-	M	±	S	-
<i>Zygopichia</i>								
<i>Z. farinosa</i> .....	M	+	H	-	H	+	S	-

\* H = heavy; M = moderate; S = slight.

† DE = de-esterification.

one species of *Debaryomyces* was negative; another yeast, *Zygothansenua californica*, was weak to doubtful.

Medium I (pH 5.0 without glucose) was the next best medium with 17 positive cultures and 4 negative. The latter group represented 2 cultures of *H. subpelliculosa*, 1 of *Candida krusei*, and 1 of *Zygothansenua californica*. On medium II (pH 5.0 plus 1.0 per cent glucose) only 2 of the 21 cultures were positive (*Hansenula anomala* NRRL-1009 and *Candida tropicalis*). None of the yeasts gave a positive test on medium IV (pH 3.5 plus 0.2 per cent glucose).

None of the 21 cultures, representing 19 species, was able to de-esterify pectin on all 4 cultural media; and

only 2 species, *H. anomala* and *C. tropicalis*, gave positive reactions on 3 of the media (I, II, III). The presence of 0.2 per cent glucose in the pectin medium at pH 5.0 appeared to stimulate the ability of the yeasts tested to bring about de-esterification; but as much as 1.0 per cent caused the reverse to be true. Yeasts capable of growing on the medium without glucose were also able, in most cases, to bring about de-esterification. Although growth was obtained by the yeasts in medium IV, and in some instances it was heavy, de-esterification was negative. The only difference between media III and IV was the lower pH level (3.5) of medium IV. It is conceivable that the medium was out of the optimum range for de-esterification if attributed to the enzyme pectinesterase; but, since most of the cultures are very oxidative types, the pH values of the media shifted toward 7 during the course of growth.

Determinations for pH were run for certain of the cultures after 30 days of incubation in pectin media I and II. In general, the cultures that gave a positive test for de-esterification of medium I also elevated the pH to 7.0 or slightly above. The same elevation in pH was obtained for certain species of *Debaryomyces*, *Hansenula*, *Endomycopsis*, and *Candida* when grown in medium II but they gave a negative test for de-esterification.

#### DISCUSSION

The basal liquid broth used in this study was a more complete medium for yeast growth than that used by Luh and Phaff (1951). Their mineral-pectin medium with glucose (0.2 per cent) was devoid of vitamins which would be considered essential for good growth of many of the species tested (Burkholder *et al.*, 1944). Furthermore, their results apparently reflect this medium deficiency inasmuch as approximately one-half of the 41 species of yeasts tested gave only poor to fair growth.

In a screening technique for pectin hydrolysis, good yeast growth would be essential and especially for the weak pectin-splitting organisms. It should also be pointed out that Luh and Phaff (1951) used 2 per cent pectin, whereas in the present study 0.5 per cent pectin was used because this was found to be a more critical level to measure the loss of a calcium pectate gel, especially for weakly positive yeasts such as the four strains of *S. cerevisiae*.

De-esterification of pectin was observed with 20 species and varieties of the yeasts investigated. Most of these were film-forming, oxidative types, capable of assimilating a wide variety of carbon compounds. Enzyme de-esterification was probably responsible for the action involved although this has not been established in the present work. Additional means of measurement would be required to demonstrate with

certainly the presence of pectinesterase. It is also possible that de-esterification was brought about by alkaline conditions produced in media as indicated by elevated pH values of 7.0 and above. However, heavy yeast growth in the pectin medium without glucose would afford presumptive evidence at least of the presence of an enzyme system capable of partially splitting the pectin molecule so it could be assimilated as a source of carbon.

The significance of the de-esterification reaction by yeasts obtained from cucumber fermentations should be mentioned. Assuming that pectinesterase is responsible and is elaborated into the brine, it is very doubtful indeed if this enzyme *per se* would have any adverse effect on the firmness of the brined cucumbers. It was reported present in brines by Bell *et al.* (1950) but was not directly associated with lack of firmness. The possible danger of pectinesterase might arise from the fact that it has been shown to speed the activity of polygalacturonase and the rate of acceleration is directly related to the degree of de-esterification of the pectin substrate (Jansen and MacDonnell, 1945; Jansen *et al.*, 1945; Matus, 1948). This would imply that before rapid destruction of the protopectin in brined cucumbers (and consequent softening) could occur, it would have to be in at least a partially de-esterified state. Microorganisms growing in the brine might be considered as a source of the de-esterifying enzyme, but they are by no means the only possible source. Pectinesterase is widely distributed in plant material (Wehmer and Hadders, 1933) and its occurrence in the cucumber has been reported by workers in this laboratory (Bell *et al.*, 1951).

The evidence presented in this study would not appear to incriminate the fermentative species of brine yeasts tested to date as being a potential source of polygalacturonase in fermenting cucumber brines. Species of yeasts belonging to the genera *Torulopsis*, *Brettanomyces*, *Zygosaccharomyces*, *Hansenula*, and *Torulaspora* were negative for glycosidic hydrolysis of pectin. Such yeasts have been shown (Etchells and Bell, 1950b; Etchells *et al.*, 1952) to be responsible for the gaseous fermentation of cucumbers under both southern and northern conditions.

Species of *Debaryomyces*, *Endomycopsis*, and *Candida* obtained from films on cucumber brines were able to de-esterify pectin, but the hydrolysis evidently did not proceed further. Also, the film yeast *Zygosaccharomyces halomembranis*, which has been repeatedly found on northern brines, was negative for de-esterification of pectin. In this respect, its behavior was consistent with the other species of the genus *Zygosaccharomyces* examined. The results to date for the species of yeasts associated with films on commercial brines demonstrate that they too are not a likely source for the softening enzyme polygalacturonase.

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#### SUMMARY

A total of 139 yeast cultures, representing 61 species and varieties in 15 genera, were investigated, by use of a simplified screening technique, for their ability to hydrolyze citrus pectin in cultural media as indicated by de-esterification and glycosidic hydrolysis. The cultures studied came chiefly from commercial cucumber brines and related brined, salted, and pickled material; 31 species came from other yeast collections.

In the following 6 genera, 20 species and varieties were capable of de-esterifying citrus pectin: *Debaryomyces*, *Candida*, *Endomycopsis*, *Hansenula*, *Rhodotorula*, and *Zygosaccharomyces*. Included in the first 3 genera named were species of film yeasts found on the surface of commercial cucumber brines (that is, *Debaryomyces membranifaciens* var. *Hollandicus*, *Endomycopsis ohmeri* and *Endomycopsis ohmeri* var. *minor*, and *Candida krusei*).

Only 6 of the 139 cultures tested gave clear-cut tests for glycosidic hydrolysis of pectin. They were *Saccharomyces fragilis*, *S. fragilis* var., and 4 strains of *Saccharomyces cerevisiae*. All came from sources other than cucumber brines.

The results to date on surface and subsurface yeasts from cucumber pickle brines give no evidence that such species would be incriminated as a potential source of the salt-stock softening enzyme polygalacturonase.

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