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Bloater Formation by Gas-forming Lactic Acid Bacteria in Cucumber Fermentations[1]

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The formation of "bloaters" (hollow stock) in cucumbers brined for salt-stock purposes at 5 to 10% salt has been associated with gaseous fermentation caused chiefly by yeasts. Recently, serious early bloater damage, not attributable to yeasts, has been observed in commercial-scale experiments on control of bloaters in overnight dill pickles brined in 50-gal barrels at 3.0 to 4.5% salt. Growth of fermentative species of yeasts was effectively controlled by the addition of 0.025, 0.05, and 0.1% sorbic acid or its sodium salt. In contrast to this, the fermenting brines showed extremely high populations of acid-forming bacteria, identified as *Lactobacillus plantarum*, *L. brevis*, and *Pediococcus cerevisiae*. The gas-forming species (i.e., *L. brevis*) constituted a high proportion of the total populations. Representative isolates from 36 barrels of overnight dill pickles were tested for their ability to produce bloaters in 1-quart jars of pasteurized cucumbers equilibrated at 4 to 5% salt, 0.25% lactic acid, and *p*H 4.0. Bloaters, identical with those made by yeast cultures, were produced in all jars inoculated with *L. brevis*. No bloaters were produced by *L. plantarum* and *P. cerevisiae*. These results suggest that the control of bloater damage in cucumber fermentations, particularly at low salt concentrations, may necessitate inhibition of gas-forming lactic acid bacteria.

Bloater damage (hollow stock) in brined cucumbers has been attributed chiefly to gaseous fermentation produced by yeasts (8, 9, 12, 18), although at times the sporadic hydrogen-carbon dioxide fermentation resulting from the growth of coliform bacteria of the *Aerobacter* genus and of closely related obligate halophilic bacteria also have been known to cause bloater formation (13). However, the hydrogen-producing bacteria are very sensitive to the acid formed during the fermentation. They are, therefore, usually found only during the very early stages of the fermentation proper, and their development is inhibited by the rapid development of the lactic acid bacteria. No other fermentations have, to our knowledge, been implicated in causing this undesirable condition of bloating—until the present report.

Our interest in investigating the possibility that another group of brine organisms, such as the gas-forming lactic acid bacteria, could produce bloaters stemmed from a set of four 1-gal (3.8 liter) samples of severely bloated "overnight" dill pickles sent to us for examination by a pickle company located in New York, N.Y. Four of every five pickles cut were "balloon"-type bloaters, meaning they had a large, single internal cavity running lengthwise of the cucumber. We were requested to try to determine the cause of the bloating and, if possible, give suggestions to eliminate or control this condition.

In the pickle industry, the overnight dill pickle is considered a specialty item, usually prepared in bulk by small packers located in large metropolitan areas of the country. The product, briefly described, usually consists of medium- to large-sized cucumbers, packed in barrels together with various spices and fresh garlic, then covered with a relatively weak brine solution containing oil of dill weed emulsion. The barrels may be stored for a few days at room temperature and then refrigerated (ca. 2 to 5 C). Under such conditions, and at equilibrated brine strengths of 10 to 15° salometer (1° salometer = 0.264% salt by weight), microbial and enzymatic activity, together with the curing process, continues at a slow rate (21). In a few months, the stored pickles may have lost

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much of their desired characteristic flavor, texture, and color, and may be bloated as well. Bloaters represent a serious economic loss to the average overnight dill packer, because they are not marketable as whole pickles. Since he packs only the one item, he has no way to utilize bloated stock, such as in cut pickle or relish, as is usually done by large pickling companies with the bloaters they grade out of their vats of cucumber brine-stock.

Refrigerated overnight dill pickles are withdrawn from storage as needed and are usually delivered to the local trade in bulk containers of various sizes. More recently, these pickles have been prepared directly in consumer-size glass containers and then distributed and retailed under refrigerated conditions. Whether made in bulk or in the jar, the fact remains that the very nature of the product makes if difficult to maintain good quality pickles for any reasonable length of time. As pointed out by Schucart (20), "The chief characteristics of these pickles are the low salt and vinegar content, the strong spicing or seasoning, (especially with respect to fresh garlic), *and their perishability*" (italics are ours).

In this paper, we report the results of our attempts to control bloater formation in overnight dill pickles by the use of the antifungal agent sorbic acid or its sodium salt. Although bloater control by use of this chemical was not successful, continued experimentation as to the reason for the failure led us directly to the important finding, presented herein, that gas-forming lactic acid bacteria are capable of producing bloaters in brined cucumbers.

MATERIALS AND METHODS

The overnight dill pickles were prepared in well-coopered, clean 50-gal (189.25-liter) wooden barrels by the two cooperating pickling plants (I and II) located in New York, N.Y. Their regular procedure was used throughout, which for plant I was as follows. Approximately 225 lb (102 kg) of no. 3 size Model variety green cucumbers [1.5 to 1.75 inches (3.8 to 4.4 cm) in diameter] were packed in each barrel together with 1 lb (454 g) of mixed spices, 1.5 lb (680 g) of crushed, fresh garlic, 2 oz (57 g) of carraway seed, and five or six dried, hot peppers. Next, each barrel was fitted with a "false" wooden head that covered the cucumbers and then about 19 gal (73 liters) of a cover brine containing 5.3% salt and oil of dill weed emulsion was added. This amount brought the brine level to about 2 inches (5 cm) above the head and about the same distance from the top of the barrel. The barrels were stored upright at room temperature (21 to 24 C) for 2 weeks before examination of the pickles.

The procedure used by plant II for preparing the pickles was as described except that the barrels were filled with large, no. 3 size, Kirby's Stays Green cucumber variety (2 inches in diameter), and then the barrels were placed on their side and a cover brine of 9.5% salt was added through the bung hole. Also, at this plant they wanted the pickles stored at room temperature only 1 week before examination.

The experimental treatment as to the addition of sorbic acid and its sodium salt was the same for both plants. Four equivalent levels of each form ot the chemical—0, 0.025, 0.05, and 0.10%—were added during the preparation of the pickles. The treatments were in duplicate or triplicate, requiring 19-barrel lots at plant I and 17-barrel lots at plant II.

The influence of the various levels of sorbic acid on the overnight dill pickle fermentation was determined by results obtained from the following physical, chemical, and microbiological tests, which have been described before. First, 100 pickles from each barrel were cut lengthwise and examined for evidence of bloating; the bloaters found were classified as "balloon," "lens," or "honeycomb" type, as illustrated earlier (15). In the balloon type, the carpels of the cucumbers separate because of gas pressure and are pressed flat toward the skin, leaving a large, single cavity. In the lens type, the gas pockets are smaller, are lenticular in shape (biconvex), and usually occur perpendicular to the long axis of the cucumber. The honeycomb type consists of a small (2- to 5-mm diameter) cavity that forms around individual, immature seeds of the cucumber. Brine samples (50 ml) were collected aseptically and shipped by air to our laboratory for analysis as to acidity (calculated as grams per 100 ml of lactic acid), *p*H, salt content, and estimates of populations of yeasts and lactic acid bacteria (14). A 10-ml portion of each brine sample was preserved with toluene and shipped to the laboratories of Union Carbide Corp., South Charleston, W.Va., for determination of the sorbic acid content.

The cultures of yeasts and acid-forming bacteria picked from the culture plates were maintained on Yeast Extract Agar and Liver Infusion Agar, respectively (17). The methods and classification systems employed for the yeast isolates were essentially those of the Dutch workers (19). Certain other techniques used in connection with the taxonomic and isolation procedures for the yeasts have been fully described (8, 12). The acid-forming bacteria were identified by conventional techniques used for lactobacilli (7) and were classified according to *Bergey's Manual*.

Cucumbers used for testing the ability of pure cultures of yeasts and lactic acid bacteria to produce bloaters under laboratory conditions were prepared as follows. Freshly harvested and washed Model variety, immature cucumbers, no. 1B size (1 to 1⅛ inches in diameter), were packed in 1-quart jars and covered with an acidified salt brine of sufficient strength to equilibrate at about 0.25% lactic acid, 4.5% salt, and *p*H 4.0. The packed and brined jars were then sealed with 66-mm diameter Vapor Vacuum-Pry Off metal closures (White Cap Co., Chicago, Ill.), pasteurized in a continuous, hot water commercial unit to an internal product temperature of 74 C for 15 min, and promptly cooled. Pasteurized cucumbers were also used for studying the effect of nine different salt levels on growth and bloater forma-

tion by *Lactobacillus brevis*. These lots were prepared as described above except that sufficient amounts of dry salt were added to a series of 1-quart jars to equilibrate at the approximate brine strengths desired (i.e., 0, 2, 4, 6, 8, 10, 12, 16, and 20% NaCl by weight).

The microorganisms used for the pure culture inoculation experiments were isolated from cucumber fermentations. Of the three species of lactic acid bacteria listed in Table 2, seven strains of *L. brevis* and one strain each of *L. plantarum* and *Pediococcus cerevisiae* were obtained from overnight dill brines during the present study; the remaining strains of these three lactic species were isolated earlier during the natural fermentation of cucumbers brined under commercial conditions for brine-stock purposes. The same was true for two of the three species of yeasts employed, *Saccharomyces rosei* and *Debaryomyces nicotianae*, whereas both strains of the remaining species, *S. delbrueckii*, came from the samples of overnight dills sent to us early in the current investigation.

For inoculation, the 1-quart jars of pasteurized cucumbers and their caps were wiped with 70% alcohol, and the area around the cap was flamed. The cap was carefully pried loose, and three or four cucumbers were removed aseptically, to provide adequate head space for a gaseous fermentation. Then, 1 ml of a 24-hr broth culture of the appropriate organism was introduced. After inoculation, the jar cap was immediately reseated. Some jar caps were punctured with a sterilized ice pick, and the culture was introduced through the small hole, which was then promptly plugged with sterile cotton. The liquid level of the jars was marked so that any change in volume of the contents of the result of fermentation could be determined.

After 11 days of incubation at 30 C, all jars were opened, and the cucumbers were examined for bloaters. Brine samples were collected from each jar and tested for acidity, *p*H, and salt content; samples were also streaked on appropriate culture media for isolation of the predominant organism present.

Results and Discussion

The brines from the four 1-gal samples of overnight dill pickles submitted for examination, which consisted of 54 to 93% balloon bloaters, contained rather high populations of both yeasts and acid-forming bacteria. The brines had an NaCl content of 3.0 to 3.2%, an acidity of 0.49 to 0.61% (as lactic acid), and a *p*H of 3.65 to 3.80, and contained 13 to 20 million live yeast cells per ml. Colony counts revealed the presence of 4.5 to 6.1 million yeast colonies per ml and 90 to 500 million colonies of acid-forming bacteria. The fermentative (gas-forming) yeast flora was found to consist of only one species, *S. delbrueckii*, which was abundant and grew readily at both 28 and 6 C. A film-forming yeast, *Pichia polymorpha*, was also isolated.

On the basis of our previous experience, it seemed possible that bloater formation in overnight dills might be controlled if development of fermentative yeast species such as *S. delbrueckii* could be suppressed.

To test this hypothesis, experimental lots of overnight dills were prepared at two commerical plants with sorbic acid or its sodium salt used to inhibit yeast growth. This chemical was chosen because, at proper concentrations, its efficacy in suppressing yeast growth in fermenting cucumber brines, while allowing the lactic acid bacteria to develop, has been well established (2–6, 10). The results of these tests (Table 1) were surprising. The overall reduction of yeast populations by the chemical additive was striking indeed, but bloater formation in the treated lots *was not controlled*. In fact, the percentage of the more important balloon and lens-type bloaters was about the same for all treatments conducted at both pickling plants. Lack of bloater control in these extensive brining tests necessitated formulation of an alternative hypothesis.

The high populations of acid-forming bacteria found in the fermenting brines of the various sorbic acid-treated lots (Table 1) were found to be comprised of three species of lactic acid bacteria; *P. cerevisiae*, *L. brevis*, and *L. plantarum*. Differential counts were not made, but the heterofermentative species, *L. brevis*, was apparently present in large numbers, and was isolated repeatedly when random colonies were picked from the culture plates reserved for identification purposes. In earlier studies (2), based on growth tests with liquid media, *L. brevis* was found to be more tolerant to 0.10% sorbic acid over a *p*H range of 5.0 to 7.0 than either *L. plantarum* or *P. cerevisiae*. At the more critical range, *p*H 4.0 to 4.5, the growth of *L. brevis* in the presence of the chemical was equal to that of *L. plartarum*, but much greater than that of *P. cerevisiae*; the latter species failed to grow at *p*H 4.0. Thus, based on these results and similar findings by Costilow et al. (4, 5), one would expect to find *L. brevis* with a high degree of frequency during the fermentation of the overnight dill pickles containing sorbic acid.

Since *L. brevis* was the only bacterial gas producer detected in the fermentations shown in Table 1, pure culture inoculation experiments were designed to test the ability of this species to produce carbon dioxide in sufficient volume to cause bloating of brined cucumbers. Two fermentative yeasts of known bloating ability were included for comparison; also, a nonfermentative yeast species together with two non-gas-forming species of lactic acid bacteria were used as negative controls. These five species are listed in Table 2.

TABLE 1. *Occurrence of bloaters in fermenting overnight dill pickles in the presence of sorbic acid*[a]

Plant no. and treatment	No. of 50-gal lots	Amt of sorbic acid		Examination of fermenting brine					Bloaters found		
				Chemical			Bacteriological				
							Colony count				
		Added	Found[b]	NaCl content	Acidity as lactic acid	pH	Yeasts	Acid-forming bacteria	Balloon and lens type	Honey-comb type	Total
		%	%	%	%		*thousands/ml*	*millions/ml*	%	%	%
Plant-I											
A	3	none	none	3.2	0.59	3.50	2,033	320	25	23	48
B	6	0.025	0.018	3.0	0.72	3.30	110	280	25	21	46
C	5	0.050	0.031	3.0	0.75	3.30	3	335	12	21	33
D	5	0.100	0.072	3.0	0.75	3.35	1	290	27	19	46
Plant-II											
A	3	none	none	4.5	0.41	3.20	100	255	31	10	41
B	5	0.025	0.014	4.0	0.44	3.20	2	190	29	5	34
C	5	0.050	0.030	4.0	0.45	3.25	9	130	31	5	36
D	4	0.100	0.071	4.5	0.41	3.30	1	190	23	4	27

[a] Values shown represent averages for each treatment shown; also, those values shown for sorbic acid include the treatments of equivalent levels of sodium sorbate. The fermenting brines and pickles from plants I and II were examined after 2- and 1-week storage at room temperature, respectively. See Materials and Methods for details on preparation of pickles at both plants.

[b] Refers to amount found in brines after 2 weeks (plant I) and 1 week (plant II). The initial amount of sorbic acid added to cucumber brines does not remain constant but, as reported earlier (1, 5), undergoes a progressive loss during the fermentation period. The rate of loss appears to be related to the brine strength employed; for example, the loss in 4.5% salt brines was at about twice the rate for brines at 9.2% (10).

TABLE 2. *Bloater formation during pure culture fermentation of pasteurized cucumbers*[a]

Microbial group and species	No. of strains tested	No. of fermentations	No. of cucumbers examined	Bloaters found			Avg brine acidity as lactic	Avg brine pH
				Balloon type	Lens type	Total		
				%	%	%	%	
Lactic acid bacteria								
Gas-forming sp.								
Lactobacillus brevis	12	30	392	67	21	88	0.56	3.60
Non-gas-forming spp.								
L. plantarum	7	10	158	0	0	0	0.98	3.25
Pediococcus cerevisiae	2	8	100	0	0	0	0.58	3.55
Yeasts								
Gas-forming spp.								
Saccharomyces rosei	1	2	28	43	39	82	0.22	4.00
Saccharomyces delbrueckii	2	2	32	56	41	97	0.32	3.95
Non-gas-forming sp.								
Debaryomyces nocitianae	1	1	17	0	0	0	0.20	4.10
Uninoculated controls	—	8	99	0	0	0	0.26	3.95

[a] See Materials and Methods for preparation of these cucumbers.

Brine turbidity developed rapidly in all inoculated jars except those seeded with *D. nicotianae*. This organism produced a luxuriant, wrinkled film on the brine surface, which is characteristic of its growth. Within 48 to 72 hr, gas was being produced actively in jars inoculated with *L. brevis*, *S. rosei*, and *S. delbrueckii*, but no gas appeared in jars seeded with *L. plantarum*, *P. cerevisiae*, or *D. nicotianae*. The presence of gas was accompanied by an increase in volume of the contents of

the jars whose lids had been punctured. There was no volume increase in the jars where the lids were not punctured, in jars where a non-gas-producing microbe was used for inoculum, or in the uninoculated controls.

When inoculated jars showing obvious gas pressure on the caps were opened, there was an immediate and copious evolution of carbon dioxide. The original liquid level of these jars changed rapidly, reaching an average increase per jar of about 100 ml in less than 5 min. Much of this increase in volume was caused by the cucumbers, which, noticeably distended by internal gas pressure during the actual bloating process, had actually increased in size. At this particular moment, cutting a balloon-type bloater with a knife resulted in a distinct popping sound. Also, these bloaters were dry inside.

In the advanced cases of bloating, the gas pressure was sufficient to separate the three carpels, representing the seed and flesh portions of the cucumber, and then press them toward the skin, leaving a single, large cavity. This type of bloating action literally forced a good portion of the liquid out of the cucumber tissue and into the cover brine, thus contributing to the increase in volume of the jar contents. If the jars were allowed to stand open for a sufficient length of time, the gas slowly diffused from the cavities and was replaced by liquid, and the brine level receded.

All fully immersed cucumbers in jars containing gas-forming organisms were severely bloated; this was true for cucumbers in jars having either punctured or intact lids. In all cases, the culture used for inoculation was recovered in abundance from the brine of the corresponding test jar. In contrast, repeated microbiological tests on samples of tissue removed aseptically from the interior of these bloated cucumbers were negative for the organisms found in such large populations in the brine from which the bloaters were removed. Uninoculated controls were negative for microbial activity.

These observations on the gaseous fermentation produced by pure cultures suggest the following mechanism for bloater formation. The fermentation gas, which is produced solely in the cover brine, diffuses into the cucumber via the brine in a supersaturated state, and is released and accumulates at a location of structural weakness inside the cucumber fruit, such as (i) where the three carpels are joined—resulting in a balloon-type bloater or (ii) in the gelatinous area around individual seeds—resulting first in the appearance of the honeycomb defect which, under continued stress of the gaseous fermentation, may develop into a typical lens-type bloater or develop even further into a combination bloater, consisting of an advanced lens and a partial balloon.

We have tested this explanation for bloater formation in the complete absence of the usual gaseous fermentation produced by microbiological activity. To do this, no. 1B size, fully cured, deacidified brine-stock cucumbers were packed into 1-quart jars and covered with the original curing brine, which had an acidity of about 0.75%, calculated as lactic, and tested 12% NaCl. An excess of sodium bicarbonate solution was then added in a single operation. The released carbon dioxide quickly supersaturated the brine, diffused into the cucumber tissue, and produced bloaters in a matter of minutes which were typical in all respects of those produced by the gaseous fermentations of microbial origin.

The results of the pure culture inoculation experiments on bloater formation are summarized in Table 2. It is apparent that *L. brevis* was able to produce bloaters in brined cucumbers. Further, the nature and extent of bloater damage by this organism was comparable in all respects to that produced by the yeasts. These laboratory tests strongly suggest that *L. brevis* produced the gas responsible for the bloating observed in the overnight dill pickles (Table 1) brined at 3.0 to 4.5% salt. This species is generally considered to be less salt-tolerant than either *L. plantarum* or *P. cerevisiae* (7, 11); even so, the results presented in Table 3 demonstrate that growth and resultant

TABLE 3. *Influence of salt on growth and bloater formation by Lactobacillus brevis in pasteurized cucumbers*[a]

NaCl content	Populations of acid-forming bacteria (millions/ml)		Balloon- and lens-type bloaters found[b]
	Maximal	Mean	
%			%
0	950	263	75
2.1	850	—	100
4.2	470	130	90
6.1	293	65	87
8.3	29	6	46
10.6	0.10	0.04	0
12.2	<0.001	<0.001	0

[a] Plate counts made at six intervals (2, 4, 6, 11, 20, and 25 days) during the fermentation period; examination for bloaters made after 28 days of incubation at 30 C; plate counts made for lots at ca. 16 and 20% NaCl were <1,000/ml throughout the fermentation period, and the same was true for uninoculated controls for each salt level. All values shown are the average for two strains of *L. brevis*.

[b] Uninoculated controls at all salt levels were negative for bloaters.

bloater formation by *L. brevis* occurred in salt concentrations as high as 8.3%, but not at 10.6%.

It does not necessarily follow that, in a highly competitive environment, such as that created by the heterogeneous natural microflora present during the fermentation of commercially brined cucumbers, *L. brevis* would predominate or even develop sufficiently at 8% salt to bring about bloater formation. Certainly, other species of lactic acid bacteria with higher salt tolerance (7), together with a number of very salt-tolerant yeasts (8, 9, 12), would be competing for the same nutritive materials; such was not the case in the pure culture fermentations (Table 3). Here, in the absence of competitive microorganisms, the inhibitory action of increasing levels of salt on populations of *L. brevis* first became noticeable at 4.2%, whereas at 6.1% it appeared to be rather pronounced.

Identification studies on the lactic acid bacteria present in actively fermenting commerically brined cucumbers, in two different areas of the country, are in agreement that three species—*P. cerevisiae*, *L. plantarum*, and *L. brevis*—can be isolated with a rather high degree of frequency (4; Borg, Etchells, and Bell, Bacteriol. Proc., p. 19, 1955; Borg, Etchells, and Bell, *unpublished data*). The same research also indicated that *L. brevis* was generally obtained much less frequently than the other two species, but no explanation was offered for this finding. To gain a meaningful insight into the prevalence or dominance of a given lactic species, such as *L. brevis*, in commercial cucumber fermentations at different brine strengths, it will first be necessary to separate the populations of lactic acid bacteria throughout their active fermentation period according to species, including any sequence of species, as has been done for the brine yeasts (8, 12).

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