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CHARACTERISTICS OF LACTIC ACID BACTERIA FROM COMMERCIAL CUCUMBER FERMENTATIONS[1]

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In commercial practice cucumbers are brined in wooden vats ranging in capacity from about 100 to 1,200 bushels. The vats, after being filled with green cucumbers, are fitted with false heads, and salt brine of a given concentration is added to a level a few inches above the head. Next, dry salt is added on the false head of the vat to maintain the initial concentration, which otherwise would become diluted by the water from the cucumbers. The initial brine strength used ordinarily ranges from about 8 to 10 per cent, depending upon the individual plant concerned. In most instances the brine concentration is gradually raised so that a holding strength of about 16 to 18 per cent is reached after about 6 weeks. Under these conditions an active acid fermentation resulting from the growth of salt-tolerant, acid-forming bacteria usually begins within a day or so after the cucumbers are brined and may continue for about 6 weeks. The preserving effect of the brine is due chiefly to the combined action of the salt and the developed acidity.

The role of the acid-forming bacteria in commercial cucumber fermentations, from the standpoint of populations occurring at various brine strengths and their relation to the principal chemical changes taking place in the brines, is probably better understood than the identity of the organisms concerned. The present study was undertaken in an effort to obtain more specific information on the nature of the predominating lactic acid bacteria involved during the fermentation of salt-stock cucumbers, under conditions typical of the industry.

EXPERIMENTAL

Particular emphasis in the current investigation has been placed on 36 isolates obtained during the 1938 season. These were isolated from the active phase of the acid fermentation of 85-bushel lots of cucumbers brined at about 5, 8, and 10.5 per cent salt concentration.[2] Information concerning the source of these

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[2] In the cucumber pickling industry, brine salinity is usually recorded in terms of degrees salometer (° sal.) as measured by a hydrometer calibrated in percentage of saturation with respect to sodium chloride (0 to 100° sal.). In the brining treatments described herein, commercial practices were followed throughout; however, for the convenience of the reader, degrees salometer have been converted to the approximate equivalent in percentage of salt by weight (e.g., 20, 30, and 40° sal. brines would approximate 5, 8, and 10.5 per cent salt brines).

cultures and other pertinent facts on brining treatment and fermentation activity are given in table 1. In addition to these cultures, 13 random isolates from six 720-bushel lots of fermenting cucumbers at various stages of activity were also studied. These lots received essentially the 10.5 per cent brine treatment outlined in table 1 for vat 14. Details concerning the origin of the cultures from these fermentations are presented in table 2.

The isolates studied were considered representative of a larger group of cultures collected over a period of several seasons from actively fermenting commercial cucumber brines. Fermentations occurred for the most part during the month of July, with brine temperatures within the range of 27 to 30 C. All vats were

TABLE 1

Origin of cultures isolated in sequence from cucumber fermentations (85-bushel lots) receiving 5, 8, and 10.5 per cent brine treatments

LOT NUMBER (1938)	BRINING TREATMENT DESIGNATION AND INITIAL CONCENTRATION THE 1ST WEEK*		RATE OF INCREASE OF BRINE CONCENTRATION PER WEEK		NUMBER OF ISOLATES	FERMENTATION PERIOD COVERED BY ISOLATIONS	MEAN PLATE COUNT OF ACID-FORMING BACTERIA DURING ISOLATION PERIOD	MAXIMUM BRINE ACIDITY AS LACTIC	MINIMUM BRINE pH
	per cent	*°sal.*	*per cent*	*°sal.*		*days*	*millions per ml*	*per cent*	
Vat 8	5.0	20	2.5	10	21	3rd to 13th†	73.1	0.78 (10th day)	3.31
Vat 13	8.0	30	2.0	8	6	3rd to 13th‡	66.2	0.60 (12th day)	3.30
Vat 14	10.5	40	1.25	5	9	5th to 13th§	4.2	0.41 (15th day)	3.60

* In the three brining treatments shown (5.0, 8.0, and 10.5 per cent), the initial brine concentration was maintained for the first week and then increased as indicated (column 3) until 15 per cent was reached. All vats were filled June 27, 1938.

† Two cultures isolated per day during the period, except one isolation on the 6th day.

‡ One culture each was isolated on the 3rd, 10th, 11th, and 12th days; two cultures on the 13th.

§ Two cultures each were isolated on the 5th, 7th, 11th, and 13th days; one culture on the 9th.

located outside and were unsheltered. The investigations were conducted at two large pickling plants located in eastern North Carolina.

All isolates were obtained by picking representative colonies from plates of nutritive caseinate agar. This medium was used for the purpose of following the populations of acid-forming bacteria in the fermentations studied. Also, it has been found adequate for the isolation of both gas-forming and non-gas-forming types of lactic acid bacteria in certain pickle products, particularly low-salt-content dills and improperly pasteurized fresh cucumber pickles, in which both types are commonly found.

The cultures were studied during the spring of 1939, and the criteria for classification included morphological observations and determination of cultural characteristics on various liquid and solid media (i.e., milk with indicator,

potato, cucumber-juice agar, cucumber-juice broth, nutrient broth, nutrient agar, nutritive caseinate agar with indicator, glucose tryptone agar with indicator, glucose agar, glucose broth, and gelatin glucose stab). Other tests included relation to oxygen, action on casein and nitrates, indole production, optimum growth temperature, action on carbon compounds, relation to salt, thermal death temperature, and carbon dioxide production. The methods employed were essentially those suggested by the work of Pederson (1930, 1936, 1938).

TABLE 2

*Origin of random cultures isolated from six cucumber fermentations (720-bushel lots) receiving 10.5 per cent brine treatment**

LOT NUMBER (AND DATE FILLED—1936)	NUMBER OF ISOLATES AND FERMENTATION AGE WHEN ISOLATED		BRINE CONCENTRATION AT TIME OF ISOLATIONS		PLATE COUNT OF ACID-FORMING BACTERIA AT TIME OF ISOLATIONS	BRINE ACIDITY AS LACTIC		BRINE pH AT TIME OF	
						At time of isolations	Maximum developed	Isolations	Minimum
	number	*day*	*per cent*	°*sal.*	*millions per ml*	*per cent*	*per cent*		
Vat 124 (7-1)	2	11th	11.4	43	3.1	0.44	0.45 (16th day)	3.60	3.60
Vat 125 (7-6)	2	5th	10.8	41	12.8	0.21	0.44 (12th day)	3.93	3.57
Vat 126 (7-1)	3	12th	11.4	43	0.2	0.50	0.54 (18th day)	3.50	3.50
Vat 3 (6-29)	1	9th	12.4	47	0.1	0.58	0.64 (15th day)	3.59	3.58
Vat 21 (7-8)	1	4th	11.1	42	0.8	0.16	0.45 (17th day)	5.18	3.67
Vat 17 (7-7)	1	4th	11.4	43	19.0	0.13	0.38 (18th day)	4.96	3.67
	2	7th	12.1	46	3.0	0.30		3.85	
	1	8th	12.4	47	1.7	0.33		3.77	

* This brining treatment was essentially the same as for vat 14 shown in table 1.

Other details, such as those concerning the preparation of the various cultural media employed, preparation of the carbon compounds used in the fermentation tests, determination of titratable acidity and pH, and other tests incident to the study have recently been described by Etchells, Fabian, and Jones (1945) and Etchells and Jones (1946). The actual species allocation was on the basis of the characteristics set forth by Pederson (1936) and by Bergey *et al.* (1939).

The results with respect to morphological, cultural, and certain of the biochemical characteristics for the cultures will not be given in detail, since they were entirely typical of those described for the *Lactobacillus* species identified. Summarized results for additional tests, such as action on carbon compounds, relation

to salt, thermal death temperature, and carbon dioxide production are presented below.

Action on carbon compounds. Definite acid fermentation is shown by the majority of strains on *l*-arabinose, glucose, *d*-galactose, lactose, fructose, maltose, *d*-mannose, raffinose, salicin, and sucrose. In general, the action on sucrose and raffinose varies more than with the other compounds in this group. Less active acid fermentation is obtained with most strains on dextrin, glycerol, mannitol, *d*-sorbitol, and *l*-xylose, as indicated by both the number of nonfermenters and the smaller amounts of acid produced. The compounds inulin, rhamnose, starch, and melezitose are fermented either not at all or, at best, only to a rather limited degree.

Relation to salt. In cucumber fermentations little or no growth is found at 15 per cent salt concentration or above. At brine concentrations below 15 per cent there is an inverse relationship between populations found and the salt concentration employed. Correspondingly lower populations and brine acidity are observed as the salt content of the brine is increased up to the inhibiting range (15 per cent). Laboratory tests on the salt tolerance of cultures may not provide a reliable index to their behavior toward salt under natural conditions. Cultures isolated from actively fermenting brines at 10 to 12 per cent salt concentration may not show growth in liquid media plus salt much above one-half the original isolation concentration.

Thermal death temperature. This is about 65 to 70 C for 15 minutes. Some strains may survive 60 C for 15 minutes, but usually they are killed by exposure to 65 C for the same time interval. Cultures from cucumber fermentations have not been observed to withstand 70 C for 15 minutes.

Carbon dioxide production. Strains not considered gas-producing in the sense that the term applies to the four gas-producing species of the *Lactobacillus* genus. The mean carbon dioxide production for 49 strains tested (with Eldredge tubes) in the present study was 5.4 per cent. About 20 per cent is the usually accepted range for members of the gas-producing species (Hucker and Pederson, 1930; Pederson, 1931, 1939).

The results with respect to the characteristics of the cultures investigated assure identification of the 36 strains from 5, 8, and 10.5 per cent brines among the non-gas-producing species of the genus *Lactobacillus*. Furthermore, it appears certain that the characteristics are distinctly more typical of those described for *Lactobacillus plantarum* (Orla-Jensen) Bergey *et al.* than for those of the remaining non-gas-producing species listed (Bergey *et al.*, 1939). On the basis of results from similar identification studies (particularly with respect to carbohydrate fermentations and carbon dioxide tests), the 13 random cultures, from six other commercial fermentations, are likewise considered as belonging to the species *Lactobacillus plantarum*.

DISCUSSION

The results of this investigation indicate that *L. plantarum* was chiefly responsible for the brine acidity of the fermented cucumbers and that other common

types of lactic acid bacteria, such as species of *Leuconostoc* or the gas-producing species of *Lactobacillus*, did not contribute materially to acid formation in the fermentations studied. The latter conclusion is based on the fact that no members of these genera were isolated either from the brines that were studied at rather close intervals during the active phase of acid fermentation or from those that were sampled at random.

In considering the inactivity of the gas-producing lactobacilli (e.g., *L. brevis*) in the brines studied, it appears likely that brining procedures using salt concentrations of 5 to 10 per cent exert a more inhibitive influence on the population development of these types than on that of the non-gas-producers (e.g., *L. plantarum*). The work of Vahlteich, Haurand, and Perry (1935) lends support to this view. They concluded that acid formation in two commercial cucumber fermentations at 10 per cent brine strength was due principally to *Lactobacillus cucumeris* (syn. *L. plantarum*); no isolates of the gas-producing lactobacilli were obtained. Further evidence of the effect of salt on the gas-producing types is found in the work on olive fermentations by Vaughn and coworkers (1943). They found these species active during the fermentation of the Sevillano variety of olives but not with the Manzanillo variety. They attributed this difference in fermentation behavior to the fact that the latter variety, in commercial practice, is brined at twice the salt concentration used for the former.

The possible influence of brine temperature and brine acidity should also be considered. The temperature requirements for certain members of both the non-gas-producing and gas-producing groups are rather similar (optimum for *L. plantarum* and *L. brevis*, 30 to 35 C). Hence, it scarcely seems plausible that the brine temperatures (27 to 30 C) encountered during the cucumber fermentations described herein could be offered as an important factor in the apparent inactivity of the gas-producing species, particularly to the extent attributed to the effect of salt. Also, the amount of brine acidity produced by the acid-forming bacteria identified as *L. plantarum* would not be considered sufficient to preclude subsequent development of gas-producing types.

The foregoing discussion has dealt principally with the influence of salt concentration on the gas-forming types of lactobacilli. In considering the failure to obtain isolates of the *Leuconostoc* genus in the cucumber fermentations studied, the influence of brine temperature as well as salt concentration must be recognized. According to the work of Pederson (1930, 1931) on sauerkraut, brine temperatures of about 30 C would not encourage rapid development of members of the *Leuconostoc* genus as compared with that of acid formers such as *L. plantarum*, even at salt concentrations well within the growth limits of both groups of organisms. As a consequence, *L. plantarum* might be expected to dominate the acid fermentations observed in the present study, both on the basis of more vigorous growth in brines at a temperature of about 30 C and as the result of higher salt tolerance as compared with the *Leuconostoc*. Although two strains of *Leuconostoc* were isolated in the studies previously referred to by Vahlteich *et al.*, these organisms were considered of minor importance in the acid fermentation.

Under certain conditions, particularly when cucumbers were put down at brine temperatures of about 20 C, an occasional fermentation has been observed by the authors to develop an abnormal viscid or syrupy type of brine. This condition, in addition to being associated with cool weather during the filling and brining operation, is also usually limited to brine treatments in the range of 5 per cent initial strength. Observations on the predominating lactic acid bacteria present in the brine have shown them to be gram-positive, gas-producing cells, ranging from short rods to spheres in shape, producing slime on sucrose media, and capable of producing more than 0.8 per cent acid (calc. as lactic) in cucumber-juice broth. In the absence of detailed taxonomic studies, these observations strongly suggest that the abnormal brine consistency occasionally observed is associated with a predominating flora of members of the *Leuconostoc* group. Such an explanation would be in keeping with the lower temperature requirements and lower salt tolerance of this group.

The possibility of influencing the character of the acid fermentation by the type of plant material employed is worthy of mention. Work in this direction would indicate that marked microfloral changes are not to be expected, provided different materials are brined or salted at similar concentrations and under similar conditions, and also contain sufficient amounts of readily fermentable carbohydrate. This is borne out in the fermentation of sauerkraut as described by Pederson (1930) and the fermentation of Sevillano olives reported by Vaughn *et al.* (1943). In these two studies the microfloral changes were remarkably similar, both with respect to the predominating types of acid-forming bacteria found and the sequence in which they occurred. Both groups of workers reported that the developed acidity resulted chiefly from three groups of acid-forming bacteria; i.e., gas-producing cocci of the *Leuconostoc* genus and non-gas-producing and gas-producing bacilli of the *Lactobacillus* genus (*L. plantarum* and *L. brevis*,[3] respectively). These occurred in the order named. In both cases, the fermentations involving these dissimilar types of material took place at relatively low salt concentrations (about 2.5 per cent for sauerkraut, and about 3 to 4 per cent for olives). In recent studies by the authors, it was observed that during the fermentation of a number of different types of vegetables (e.g., corn, peas, green beans, and okra) the principal influence on the acid-forming bacteria was exerted by the salt concentration used in the preservation treatment rather than by the type of material studied.

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[3] Referred to by Pederson (1930) as *L. pentoaceticus*. In accord with later work by Pederson (1938) this species is now considered identical with *L. brevis* (Bergey *et al.*, 1939).

SUMMARY

Identification studies on 49 cultures of lactic acid bacteria occurring during the acid fermentation of salt-stock cucumbers, under conditions typical of the industry, are reported.

Thirty-six of the cultures were isolated during the active phase of acid formation from fermentations maintained at about 5, 8, and 10.5 per cent salt concentration for 1 week, after which the brine strength was gradually increased. These isolates gave characteristics typical of those described for *Lactobacillus plantarum* (Orla-Jensen) Bergey *et al.* and were allocated to this species.

The remaining 13 cultures were isolated at random from six cucumber fermentations at brine concentrations ranging from about 11 to 12.5 per cent salt. These isolates were also considered to belong to the species *Lactobacillus plantarum.*

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