ISOLATION AND IDENTIFICATION OF A STRAIN OF *BACILLUS SUBTILIS* CAUSING PICKLE SPOILAGE

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Arnold Lester Demain

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THESIS

This is to certify that the

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ISOLATION AND IDENTIFICATION OF A STRAIN OF *BACILLUS SUBTILIS* CAUSING PICKLE SPOILAGE

By

Arnold Lester Demain

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INTRODUCTION

Spoiled pickles are a problem that has faced the industry for many years. The spoilage of salt stock pickles comes from six months to a year after the cucumbers have been placed in tanks for salting. In the case of processed dills, softening of the pickle is also a problem. Workers who have investigated the problem have attributed softening to the decomposition of pectic materials in the middle lamella of the cucumber -- the change of insoluble protopectin to soluble pectin by the action of bacterial enzymes.

PRESENT INVESTIGATION

The present investigation has to do with two different lots of pickles. The first batch arrived from the state of Delaware and contained two jars of soft salt stock pickles and two jars of processed dills made from soft salt stock. The receiving stations for the original cucumbers were within 25 miles of the salting station. The cucumbers were trucked to the salting station within two to three hours after being received. They were placed into a 30 degree brine and held until the acidity had reached 0.5 percent determined as lactic acid. At this point, the salt was raised to 50 degrees salometer within the next five days. The cucumbers were held at this salinity. Specific data for salinity, acidity and temperature from one of the spoiled tanks is shown in Table I.

The soft processed dills came from the State of Michigan. They arrived in four jars, each pair representing a different batch. One batch was processed on November 29th, and the other on December 2nd. Each run contained approximately 45 barrels with two spoiled and 43 good barrels. The process consisted of three freshening tanks, each holding 15 barrels. The dill brine came from one large tank; the same brine was used for all 45 barrels.

LITERATURE REVIEW

Van Tieghem (1879) considered that Bacillus amylobacter was responsible for the decomposition of vegetable tissue.

The action of Bacillus mesentericus vulgatus was studied by Vignal (1889). He found that the organism secreted a ferment which dissolves the middle lamell of potato tubers without dissolving the cell walls, thereby dissociating the cells.

Aderhold (1889) reported that Bacterium coli was the cause of pickle softening.

Kossowicz (1908) reported that the Bacillus mesentericus group, not Bacillus coli, was responsible for softening.

The pectase enzyme produced by Bacillus carotovorus and other soft rot organisms was studied by Jones (1909). This enzyme was capable of softening carrots very quickly by dissolving out the middle lamella, leaving the cells free.

Rahn (1913) reported the factors involved in salt stock spoilage. He concluded that acid would inhibit very quickly the growth of proteolytic bacteria while salt alone could not always do this.

LeFevre (1919) concluded that cucumber softening was caused by many organisms including the cellulose destroyers, the soft-rots, and the spore-bearing aerobes. The only organisms isolated that were capable of living in high salt concentrations were Bacillus vulgatus and Bacillus mesentericus fuscus. In the relation of salt to the

TABLE I

RECORD OF TANK CONTAINING SPOILED SALT STOCK PICKLES FROM DELAWARE

DATE	SALINITY[1] top	SALINITY[1] bottom	ACIDITY[2]	BRINE TEMP. °F.	AIR TEMP. °F.	PICKLES ADDED in lbs.	SALT ADDED in lbs.
July 6						3260	500
7						23978	3100
8						4322	500
9	28	37	1.1	76	74		
10	27	35	1.3	65	67		
12	33	36	2.1	66	69		
13	53	36	3.1	75	85		
14	30	37	4.1	74	87		
15	29	35	5.6	75	78		1200
16	42	43	5.7	72	76		1000
17	47	49	5.6	74	76		
19	51	52	5.7	76	78		
20	39	51	5.8	77	87		150
21	43	50	5.8	77	80		200
22	51	51	5.8	78	84		
23	50	51	5.7	79	80		
24	48	50	5.8	79	78		150
26	52	51	5.9	74	78		
27	53	55	5.8	78	85		
28	52	53	5.7	78	82		
29	52	52	5.8	79	83		
30	52	52	5.8	78	80		
31	52	52	6.0	78	80		
Aug. 2	51	51	5.8	78	77		
3	50	52	5.9	78	79		
4	47	50	5.9	78	78		
5	49	50	5.9	75	76		50
6	48	50	5.8	72	64		50
7	50	51	5.8	75	68		
9	53	53	5.8	74	69		
10	52	53	5.7	73	68		50
11	52	52	5.5	75	72		
12	52	52	5.7	75	74		
13	52	52	5.6	74	74		
14	52	53	5.7	74	74		33.5

[1]Expressed in salometer degrees.
[2]Expressed in grains determined as lactic acid.

Continued next page

TABLE I - Continued

DATE	SALINITY[1] top	SALINITY[1] bottom	ACIDITY[2]	BRINE TEMP. °F.	AIR TEMP. °F.	PICKLES ADDED in lbs.	SALT ADDED in lbs.
Aug. 16	51	51	5.8	72	68		
17	52	52	5.7	73	70		
18	53	53	5.8	74	70		
19	50	52	5.9	73	70		
20	51	51	5.7	73	70		
21	48	50	6.3	73	68		150
23	52	52	6.1	73	70		
24	52	52	6.0	74	72		
25	53	53	5.6	74	72		
26	53	53	5.8	75	77		
27	53	53	5.8	78	78		
28	53	53	5.6	80	83		50
30	53	53	5.3	78	79		
Sept. 1	53	53	5.8	72	66		100
3	53	53	5.7	71	72		
16	54	54	5.1				
24	51	54		74	75		50
Oct. 11	51	54	5.3	68	72		100
28	55	55	5.2				
Nov. 12	54	55	5.7				
30	52	55	5.2	50	48		200
Dec. 17	56	56	5.5	50	50		500

[1]Expressed in salometer degrees.
[2]Expressed in grains determined as lactic acid.

preservation of cucumbers, the critical point was regarded as between seven and eight percent.

Dill pickle spoilage was investigated by Joslyn (1928). He concluded that softening was due to a bacterial cause. He noticed that in a brine of pH 3.00 - 3.10, slipperiness increased. Since this pH was lower than that tolerated by Bacillus vugatus in LeFevre's work, Joslyn suggested that enzymes secreted by this organism may act at a lower pH than that tolerated by the organism itself.

Lesly and Cruess (1928) studied dill pickle spoilage and found that high initial acidity favored softening and that brine from spoiled pickles had a higher acidity and lower pH than brines of normal pickles. They suspected that the softening may be due to the high acidity which would cause the splitting of hemicelluloses or pectin. They also observed that softening did not all occur during fermentation but increased with length of storage. The larger pickles were less prone to become soft than smaller ones.

Fabian, Bryan, and Etchells (1932) studied sections of normal cured cucumber pickles and pickles in different stages of decomposition. They observed certain structural differences between the various lots of pickles. The most obvious histological change that had occurred in the soft pickles as compared to the normal pickles was in the intercellular spaces where there was a conspicuous lack of cementing material after the pickles had started to soften. This condition progressively increased until there was no longer any material left in the middle lamella.

After what was assumed to be the pectic material had disappeared, the cell wall was attacked until finally all the parenchymatous cells were gone. The most resistant parts of the cucumber were the seeds and vascular bundles.

Flax retting was studied by Trevethick, Robinson, and Snyder (1928). They reported that the Bacillus mesentericus-megatherium group caused retting by dissolving out the pectic substances. The optimum pH was found to be seven.

Hof (1935) studied bacterial life in strong brines. Pure cultures of "normal" bacteria originating from a saltless environment tolerated only a relatively low amount of salt in their culture liquid. Taking into account that bacteria as occurring in nature may adapt themselves better to a life under abnormal conditions than pure cultures, enrichment cultures were made for various bacterial groups in media with increasing percentages of salt and with inoculum of garden soil. Under these conditions, bacteria which attacked pectin under aerobic conditions grew in solutions containing up to 18 percent salt in comparison with a pure culture that grew only up to a six percent salt concentration.

Fabian and Johnson (1938) studied the bacteriological, zymological, histological and chemical changes in spoiled pickles. They found that the Bacillus mesentericus fuscus group produces a protopectinase which can cause pickle spoilage rapidly. This protopectinase becomes active after the first day's growth of the organism, and under favorable

conditions, reaches its maximum activity in six days. The histological and chemical studies proved that the softening of pickles was due to a change in the insoluble protopectin to soluble pectin by bacterial enzymes. Also acids, heat, and enzymes were found to increase the susceptibility of the pickles to softening, either immediately as by cooking, or over a long period of time with a weak acid.

Faville and Fabian (1948) found that bacteriophage races which lysed Lactobacillus plantarum cultures was isolated from soil where the cucumbers had grown but not from water or from genuine dill pickle brines in which spoilage had occurred six to eight months previously. In studying the antibiotic effect of aerobic sporogenic bacteria commonly found in soil and in fermenting cucumber vats, it was found that these organisms were capable of producing substances which inhibited greatly the growth of Lactobacillus plantarum, the organism chiefly responsible for cucumber fermentation. The two organisms capable of this action, Bacillus mesentericus fuscus and Bacillus vulgatus, were also able to elaborate pectin-hydrolyzing enzymes. In Eh studies, it was found that the six species of aerobic spore formers from spoiled pickles reduced the oxidation-reduction potential of the medium to a considerable degree while the lactobacilli had little effect on the potential. When the NaCl content of the medium was increased, the time required by the organism to reduce the potential of the medium to a level at which multiplication could take place was also increased. The effect of lactic acid was similar and when both agents were added together, the results

were additive. This indicated that the salt tolerance of these organisms decreases with increasing concentrations of lactic acid. Most of the spoilage organisms could be induced to grow in significantly higher concentrations of salt if the oxidation-reduction potential of the medium was reduced to the minimum level established by the organism during normal growth.

Fabian and Faville (1949) isolated and identified a mold, Oospora lactis, which was the causative agent in two cases of pickle spoilage. In one case, the organism developed during the freshening process of salt stock when the period of freshening was too long. In the other case, the organism caused the damage in processed dill pickles due to the use of dirty barrels.

EXPERIMENTAL PROCEDURE

Upon arrival, the jars of salt stock and process dills were analyzed bacteriologically and chemically. The bacteriological procedure consisted of plate counts on three media. Tryptone-glucose-yeast extract agar (TGYE) was used to grow and differentiate peptonizers, acid bacteria and inert bacteria. Peptonizers were distinguished by a clear zone around the colony; acid bacteria by turning the medium yellow; and inert bacteria by leaving the medium purple. The total count was also determined by this medium. Its composition is as follows:

Tryptone	10 grams
Yeast extract	5 grams
Beef extract	3 grams
K_2HPO_4	1 gram
Glucose	1 gram
Agar	15 grams
Dist. water	1000 ml.
Brom-cresol-purple	2 ml. of 1.6% solution

1 ml. sterile skim milk per plate added when plates are poured.

Potato dextrose agar (PDA) acidified to a pH of 3.5 with tartaric acid was used for yeast counts. Its composition is as follows:

Infusion from potato	200 grams
Dextrose	20 grams
Agar	15 grams
Dist. water	1000 ml.

Tomato juice agar was used to determine the count of lactic acid bacteria. Its composition is as follows:

Tomato juice (400 cc)	20 grams
Peptone	10 grams
Peptonized milk	10 grams
Agar	11 grams
Dist. water	1000 ml.

The TGYE and tomato juice agar plates were incubated for two days at 32° C. while the potato dextrose agar plates were incubated at room temperature for five days.

The chemical analysis consisted of acid, salt, and pH determinations. The acid was determined with 0.1666 N NaCH and two drops of a five percent alcoholic solution of phenol-thphalein. The direct reading was in percent acetic acid which, when multiplied by ten, gives grains of acetic acid. The salt was determined with $AgNO_3$ solution using 0.5 percent of a 95 percent alcoholic solution of dichlorofluorescein. This read directly as percent salt. The pH was determined electrometrically by a Beckman pH meter.

After the plates were counted, typical organisms were fished and put through a purification procedure. This procedure consisted of streaking the cultures and fishing isolated colonies seven times. The purification was carried out on beef extract agar, then transferred to beef extract agar slants. The bacterial cultures were then put through identification tests as outlined in "Aerobic Mesophilic Sporeforming Bacteria" by Smith, Gordon and Clark.

Microscopic Examination of bacteria

Gram stains of 24 hour cultures incubated at 35° C. on beef extract agar were made on the 16 bacterial cultures.

Macroscopic Examination of bacteria

Growth on nutrient agar was observed on slants incubated at 35° C. for four days. Growth in nutrient broth was observed after incubation at 35° C. for four days.

Physiological Examination of bacteria

Casein hydrolysis was shown on milk agar plates prepared by mixing equal quantities of sterile skim milk and sterile two percent agar, both cooled to 45° C. before mixing. After solidifying, cultures were streaked on the plates and observed for clearing of the casein.

The ability to grow at pH 6.0 was determined on slants of nutrient agar.

The reduction of nitrates to nitrites was made on cultures grown in nutrient broth plus 0.1 percent KNO_3 for four days. To five ml. of the culture was added 0.5 ml. of a one percent solution of KI, and after mixing, one drop of concentrated H_2SO_4. If nitrates were formed a blue color was the result. If there was much nitrite, a heavy blue precipitate resulted and gas was evolved.

The production of urease was demonstrated by the growing of cultures on slopes of nutrient agar and testing for urease after five days. The growth was washed off with two ml. of distilled water and divided equally between two clean test tubes. A drop of phenol red indicator was added to each and the reaction brought to pH 7.0 by a few drops of very dilute HCl or NaOH. Approximately 0.02 gm. of crystalline urea was mixed with the suspension in one tube and the other was kept as a control. If urease was present the suspension with the urea became very alkaline in a few minutes.

The hydrolysis of starch was determined by streaking plates of nutrient agar containing one percent potato starch that had been added

after filtering. After incubating for one day, the plates were flooded with 95 percent alcohol. If the starch remained unchanged the medium became white and opaque but if it was hydrolyzed a translucent zone appeared around the growth.

For the fermentation studies an ammoniacal medium was used. The formula was as follows:

$NH_4H_2PO_4$	1.0 gram
KCl	0.2 gram
$MgSO_4$	0.2 gram
Agar	13.0 gram
Dist. water	1000.0 ml.

The pH was adjusted to 7.0 and 12 ml. of a 0.04 percent solution of brom-cresol-purple was added as the indicator. The solution of the test carbohydrate was then sterilized separately by filtration through a Seitz filter. The basal medium was autoclaved and the filtered carbohydrate solution added aseptically to it (one gram carbohydrate in 25 ml. distilled water added to 175 ml. basal medium resulting in a 0.5 percent concentration of carbohydrate). Five ml. were pipetted into each tube and slants were made. These were streaked and stabbed in the butt and incubated at 35° C. Observations were made at 3, 8, 15 and 21 days.

The utilization of citrate was demonstrated on the following medium:

Na citrate	2.0 grams
NH_4NO_3	2.0 grams
KH_2PO_4	0.5 gram
Agar	13.0 grams
Tap water	1000.0 ml.
0.04% Phenol red soln.	10.0 ml.

pH adjusted to 6.8 before addition of indicator

These slants were observed for growth and reaction after four days of incubation.

The Voges-Proskauer reaction was demonstrated on the following medium:

Proteose-peptone	7.0 grams
Glucose	5.0 grams
NaCl	5.0 grams
Dist. water	1000.0 ml.

Five ml. portions in 18 mm. tubes were inoculated and incubated at 32° C. for two and four days. The presence of acetylmethylcarbinol was demonstrated by the appearance of a red color after the addition of an equal volume of a 40 percent NaOH solution and a few milligrams of creatine and shaking.

Gelatin hydrolysis was determined on plates of nutrient agar containing 0.6 percent gelatin. These were streaked and incubated at 35° C. for one day. This is slight modification of the procedure of Smith, Gordon and Clark. The plates were then covered with nine ml. of the following test solution:

Dist. water	100 ml.
Conc. HCl	20 ml.
$HgCl_2$	15 grams

This reagent formed a white opaque precipitate with the unchanged gelatin and left a clear zone where the gelatin was hydrolyzed.

The results of all identification tests were checked with Bergey's Manual of Determinative Bacteriology, sixth edition. The isolates were placed in groups resembling certain species and one member from each

group was selected to determine the protopectolytic properties of each group. The media used was that suggested by Fabian and Johnson in 1938.

Beet Sugar Molasses Medium

100 parts beet molasses
100 parts monobasic ammonium phosphate
10 parts urea
Diluted to 5° Brix with tap water

The organisms selected to represent the groups were inoculated into flasks containing 330 ml. of medium. Into another flask was inoculated the organism Bacillus mesentericus fuscus, a known pectolytic organism. A control flask was also incubated with no inoculum. The flasks were incubated for six days at 32° C. and then filtered through Buchner funnels to remove sediment. The filtered media were then placed in sterile jars, each jar containing three intact salt stock pickles freshened to 20 degrees salometer. The medium was layered with toluene to prevent bacterial action and the jars incubated at 32° C. They were observed for signs of spoilage at 1, 4, 6, 13 and 44 days.

RESULTS

The results of the chemical and bacteriological analyses of the four samples of processed dills (samples 1, 2, 3 and 4) are given in Table II. From these four samples, the following organisms were isolated:

From TGYE agar

W4.....raised dull inert colony

W5.....colony appearing as sporeformer

W6.....small colony appearing as sporeformer with clear zone

W7.....pinpoint glistening colony

From Tomato juice agar

W8.....large round dull colony

W9.....large round dull colony (duller than W8)

W10....colony appearing as sporeformer

W11....small glistening colony

Analysis of the four samples of spoiled salt stock (samples 5, 6, 7 and 8) gave the results shown in Table III. The following organisms were isolated:

From TGYE agar

L1T....from sample 5

L2T....from sample 6

L4T....from sample 8

From Tomato juice agar

L4JS...from sample 8

L4JL...from sample 8

The results of microscopic, macroscopic and physiological examinations of the isolates are presented in Tables IV, Va and b, and VI respectively. The results given in Table VI are those observed at the final reading. During purification, isolate W6 was separated into two organisms -- W6b and W6p. Also two other isolates -- W12 and W13 -- were separated.

On comparison of all identification data with Bergey's Manual, the isolates were placed into four separate groups. Each group, with the organism it most closely resembles, is shown in the following:

Group	Isolates	Organism
I.	W11, L1T, L4T, L4Js	Bacillus cereus (var. mycoides)
II.	W4, W5, W6b, W6p, W7, W8, W9, W12, W13, L4JL	Bacillus subtilis
III.	L2T	Bacilis pumilis
IV.	W10	Doubtful. May be a B. subtilis-B. pumilis intermediate

After growth for six days in Beet Molasses medium, growth appeared as follows:

Group I...Slight ring on surface.

Group II...Heavy wrinkled dry growth on surface.

Group III...Light membraneous growth on surface.

Group IV...Light membraneous growth on surface.

B. mesentericus fuscus...Heavy wrinkled greasy growth on surface.

Control...Clear.

The results of each group on freshened intact salt stock are as follows:

Group	1 day	4 days	6 days	13 days	44 days
I.	-	-	-	-	-
II.	-	-	soft	soft	soft
III.	-	-	-	-	-
IV.	-	-	-	-	-
V.	-	-	-	soft	soft
VI.	-	-	-	-	-

DISCUSSION

Considering the Michigan processed dills first, the chemical analysis shows a definite lack of sufficient acidity. The highest acidity of any of the four samples was 4.0 grains and one sample was as low as 2.5 grains. The minimum for process dills should be six grains. A recent survey of American and Canadian pickle packers showed an average of 8.5 grains used in the manufacture of processed dills. This lack of acidity was probably the cause of the large bacterial populations growing in the samples as shown by the TGYE agar results. Of the eleven isolates from these processed dills, nine were found to be of the group that showed pectin-hydrolyzing activity which is the reason why the pickles became soft.

Of the isolates obtained from the Delaware samples, only one showed pectin-hydrolyzing activity. It originally was isolated from sample eight which contained processed dills made from soft salt stock. This same sample, six months later, contained nothing more than a mushy liquid since the cucumbers completely disintegrated. On the other hand, the samples of salt stock still retain their shape, even though they were soft. This indicates the possibility that the organisms that caused the spoilage of the salt stock may have been killed or the enzyme inactivated or both after the pectin breakdown occurred in the salt stock. This may have been the reason for the lack of success in isolating the causative organism from the salt stock samples. Another possibility is that the salt stock was softened by another organism and the

causative organism isolated in this work was picked up during the freshening process.

Examination of the salting data of the spoiled tank shows that for five days after the tank was closed and eight days after it was started, salinities below 40° were present in parts of the vat. This is significant, as spoilage may easily result in such low salt concentrations when temperatures of 75° F. and above are reached. These first few days are the most important days of the salting procedure. If pectin-hydrolyzing enzymes are elaborated during these days, there is a very good chance of the cucumbers becoming soft over a period of time since it requires a long time for a small amount of enzyme to work.

Many pickle packers use an acidity of six grains determined as acetic acid in manufacturing processed dills. This has been accepted as the minimum for this type of product. However, the fact that the causative organism was so active in sample eight which had an acidity of 6.5 grains sheds new light on the problem of spoilage. Possible solutions are raising the acidity or pasteurization of processed dills. Some packers pasteurize this product but there are many who do not. The accepted procedure of 71.1° C. (160° F.) for 20 minutes should be enough to inactivate the destructive enzyme. Although the specific enzyme hydrolyzing the pectic materials of the cucumber has not been purified and analyzed, the deactivation times and temperatures for pectin-hydrolyzing enzymes of other sources have been determined by other workers. From their studies, it seems reasonable to assume that the pasteurization

temperature and time used in the industry today is sufficient to deactivate the enzyme. Further work is needed along these lines.

The fact that a strain of Bacillus subtilis showed pectin-hydrolyzing activity in this work is not as startling as it may seem. The organism has been used in the flax retting industry where it is known to hydrolyze pectic materials between the fibers. Previous workers have shown that it does not spoil pickles, but it must be remembered that the classification of the Bacillus group has been a confused one. Organisms called B. subtilis in the past may not be B. subtilis at all (according to present day classification). For instance, in 1948, Faville and Fabian found Bacillus vulgatus to be capable of pectin-hydrolysis, however, today this organism is regarded as a stage in the growth of B. subtilis. The organism called B. subtilis in this paper checks well with the sixth edition of Bergey's Manual in all features examined except the lactose and arabinose fermentations.

There are four main types of spoiled pickles encountered in the industry. They are classified as follows:

(a) The mushy type which usually starts out as a slippery pickle, called a "slip", and continues until the whole pickle is mushy.

(b) The cheesy type which is not slippery but crumbles throughout when pressure is applied.

(c) The "watery" type in which the inside is watery while the outside skin is still intact. This is not found with the

black spine pickling variety but in the thick skinned, white spine, slicing type of pickle and is undoubtedly the same as the cheesy type (b) except the skin is so thick that it withstands the hydrolysis longer. Eventually it will break down.

(d) Loss of firmness or turgidity. A flabby pickle due to physiological factors rather than biochemical changes as in the three above mentioned types.

The type encountered in this work was the cheesy variety. This is the most frequent type of spoilage found. It is not known whether these types are caused by different factors or are merely different stages in the softening of a pickle.

SUMMARY

Two samples of soft salt stock and two samples of processed dills were received from Delaware and four samples of processed dills were received from a Michigan packer. All were of the "cheesy" type of spoilage. The eight samples were analyzed chemically and bacteriologically. Typical organisms were fished, purified and identified. The organisms isolated resembled Bacillus cereus var. mycoides, Bacillus pumilis and Bacillus subtilis. The only group showing pectin-hydrolyzing action on freshened intact salt stock pickles was the one resembling B. subtilis.

CONCLUSIONS

1. The probable reason for spoilage of the Michigan process dills was the low acidity. The four samples contained acidities of 2.5, 3.0, 3.0 and 4.0 grains. According to a recent survey the average for the industry is 8.5 grains.

2. As for the Delaware salt stock, spoilage probably resulted from the lack of sufficient salt concentrations in parts of the tanks during the first few days of salting. A 40° salinity is advisable in hot climates and going below this is dangerous. The fact that the causative organism thrived in an acidity of 6.5 grains in the processed dills shows how potent it is. Possible solutions are raising the acidity or pasteurization to deactivate the enzyme.

TABLE II

CHEMICAL AND BACTERIOLOGICAL ANALYSIS OF FOUR SAMPLES OF SPOILED PROCESSED DILLS FROM MICHIGAN

Chemical Analysis

Sample	NaCl	Acidity as acetic	pH	product
1.	3.9%	4.0 grains	3.45	proc. dills
2.	5.2%	2.5 grains	3.50	proc. dills
3.	5.4%	3.0 grains	3.55	proc. dills
4.	5.3%	3.0 grains	3.55	proc. dills

Bacteriological Analysis
(in organisms per ml. brine)

Sample	TGYE	PDA	Tomato Juice agar
1.	spreaders	297,000	280,000
2.	9,400,000 (inert)	185,000	10,100,000
3.	7,300,000 (inert)	144,000	5,600,000
4.	15,300,000 (inert)	237,000	spreaders

TABLE III

CHEMICAL AND BACTERIOLOGICAL ANALYSIS OF TWO SAMPLES OF SPOILED SALT STOCK AND TWO SAMPLES OF PROCESSED DILLS MADE FROM SPOILED SALT STOCK FROM DELAWARE

Chemical Analysis

Sample	NaCl	Acidity as acetic	pH	product
5.	18.7%	2.0 grains	3.5	salt stock
6.	18.8%	2.0 grains	3.6	salt stock
7.	8.8%	6.5 grains	3.1	proc. dills
8.	8.8%	6.5 grains	3.2	proc. dills

Bacteriological Analysis
(in organisms per ml. brine)

Sample	TGYE	PDA	Tomato Juice agar
5.	1190 inert; 180 pept.	4118	0
6.	1360 inert; 170 pept.	5900	18,500
7.	0	0	0
8.	330 peptonizers	2850	3,000

TABLE IV

MICROSCOPIC EXAMINATION OF BACTERIA ISOLATED FROM SPOILED PICKLES

W4	Gram variable rods; occurring singly, in pairs and short chains; round ends; lu x 2.3 - 3.0u; spores central, subterminal; some spores spherical 1.lu in diameter, some oval 1.0u x 1.8u; sporangia not definitely swollen.
W5	Gram - rods; occurring singly, in pairs and short chains; round ends; lu x 2.3 - 3.0u; spores central, subterminal; some spores spherical 1.lu in diameter, some oval 1.0u x 1.8u; sporangia not definitely swollen.
W6b	Gram - rods; occurring singly, in pairs and short chains; round ends; 1.0 x 2.3 - 3.0u; spores central, subterminal; some spores spherical 1.lu in diameter, some oval 1.0u x 1.8u; sporangia not definitely swollen.
W6p	Gram variable rods; occurring singly, in pairs and short chains; round ends; lu x 2.3 - 3.0u; spores central, subterminal; some spores spherical 1.lu in diameter, some oval 1.0u x 1.8u; sporangia not definitely swollen.
W7	Gram variable rods; occurring singly, in pairs and short chains; round ends; lu x 2.3 - 3.0u; spores central, subterminal; some spores spherical 1.lu in diameter, some oval 1.0u x 1.8u; sporangia not definitely swollen.
W8	Gram - rods; occurring singly and in pairs; round ends; 1.0u x 3.5u; spores few, subterminal; oval 1.0u x 1.4u; sporangia not definitely swollen. Some long chains noted.
W9	Gram - rods; occurring singly and in pairs; round ends; 1.0u x 2.7u; few spores, subterminal; oval 1.0u x 1.4u; sporangia not definitely swollen.
W10	Gram - rods; occurring singly and in pairs; round ends; 1.0 x 2.3 - 3.3u; spores subterminal; oval 1.0u x 1.5u; sporangia not definitely swollen.
W11	Gram - rods; occurring in long and short chains, in pairs and singly; round ends; 1.3u x 4.5u; spores very prominent, subterminal; oval 1.3u x 1.6 - 2.2u, spherical 1.3u in diameter; sporangia not definitely swollen.

Continued next page

TABLE IV - Continued

W12	Gram - rods; occurring singly and in pairs; round ends; 1.1u x 3.3u; spores central, subterminal; oval 1.1u x 1.4u, cylindrical, diameter 1.1u; sporangia not definitely swollen.
W13	Gram - rods; occurring singly and in pairs; round ends; 1.0u x 3.3u; spores few, central, subterminal; cylindrical, diameter 1.0u; sporangia not definitely swollen.
L1T	Gram - rods; occurring in long and short chains, in pairs and singly; round ends; 1.3u x 4.5u; spores very prominent, subterminal; oval 1.3u x 1.6 - 2.2u, spherical 1.3u in diameter; sporangia not definitely swollen.
L2T	Gram - rods; occurring singly and in pairs; round ends; 1.1u a 3.3u; spores central, subterminal; oval 1.1u x 1.4u, cylindrical, diameter 1.1u; sporangia not definitely swollen.
L4T	Gram - rods; occurring in long and short chains, in pairs and singly; round ends; 1.3u x 4.5u; spores very prominent, subterminal; oval 1.3u x 1.6 - 2.2u, spherical 1.3u in diameter; sporangia not definitely swollen.
L4JS	Gram - rods; occurring in long and short chains, in pairs and singly; round ends; 1.3u x 4.5u; spores very prominent, subterminal; oval 1.3u x 1.6 - 2.2u, spherical 1.3u diameter; sporangia not definitely swollen.
L4JL	Gram - rods; occurring singly; round ends; 0.8u x 2.3u; few spores, subterminal; oval 0.8u x 1.1u; sporangia not definitely swollen.

TABLE V a.

CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM SPOILED PICKLES ON NUTRIENT AGAR

Org.	Growth	Form	Elevation	Lustre	Topography	Optical characters	Chromogenesis	Consistency
W4	moderate	echinulate	raised	glistening	contoured	translucent	cream	viscid
W5	moderate	echinulate	raised	glistening at edge	contoured	translucent	cream	viscid
W6p	abundant	echinulate	raised	glistening at edge	contoured	translucent	cream	viscid
W6b	abundant	arborescent & filiform	raised	glistening	smooth	translucent	cream	viscid
W7	abundant	echinulate	raised	dull	contoured	translucent	white	viscid
W8	abundant	echinulate	raised	glistening	contoured	translucent	white	viscid
W9	moderate	echinulate	raised	dull	contoured	translucent	white	viscid
W10	abundant	rhizoid	raised	glistening	smooth	translucent	white	viscid
W11	abundant	arborescent	raised	glistening	verrucose	translucent	white	butyrous
W12	abundant	echinulate	raised	glistening	contoured	translucent	white	viscid
W13	abundant	echinulate	raised	glistening	contoured	translucent	white	viscid
L1T	abundant	arborescent	raised	glistening	verrucose	translucent	white	butyrous
L2T	abundant	rhizoid	raised	glistening	smooth	translucent	white	viscid
L4T	abundant	arborescent	raised	glistening	verrucose	translucent	white	butyrous
L4JS	abundant	arborescent	raised	glistening	verrucose	translucent	white	butyrous
L4JL	abundant	eschinulate	raised	dull	contoured	translucent	white	viscid

TABLE V b.

CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM SPOILED PICKLES IN NUTRIENT BROTH

Org.	Surface	Clouding	Odor	Sediment
W4	pellicle	clear	absent	scant, granular
W5	pellicle	clear	absent	scant, compact
W6p	pellicle	clear	absent	scant, flocculent
W6b	pellicle	clear	absent	scant, flocculent
W7	pellicle	clear	absent	scant, flocculent
W8	pellicle	clear	absent	scant, compact
W9	greasy pellicle	clear	absent	scant, compact
W10	ring	slight	absent	scant, compact
W11	ring	slight, fluid turbid	absent	abundant, viscid on agitation
W12	pellicle	clear	absent	scant, compact
W13	wrinkled pellicle	clear	absent	absent
L1T	ring	fluid turbid	absent	abundant, compact
L2T	pellicle	clear	absent	scant, compact
L4T	ring	fluid turbid	absent	abundant, compact
L4JS	ring	fluid turbid	absent	abundant, compact
L4JL	pellicle	clear	absent	absent

TABLE VI

PHYSIOLOGICAL REACTIONS OF BACTERIA ISOLATED FROM SPOILED PICKLES*

									Fermentation Reactions[1]													
	Casein Hydrolysis	Gelatin Hydrolysis	Starch Hydrolysis	Nitrate Reduction	Urease Production	Citrate Utilization	Growth in pH 6.0	Voges-Proskauer	Fructose	Lactose	Sucrose	Maltose	Galactose	Raffinose	Arabinose	Xylose	Mannose	Mannitol	Glucose	Glycerol	Rhamnose	Inulin	Dextrin
W4	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W5	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W6b	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W6p	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W7	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W8	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W9	P	P	P	P	O	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W10	P	P	O	P	O	P	P	P	P	O	P	O	P	P	O	P	P	P	P	P	O	O	O
W11	P	P	P	P	O	P	P	P	P	O	O	P	O	O	O	O	O	O	P	P	O	O	P
W12	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W13	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
L1T	P	P	P	P	O	P	P	P	P	O	O	P	O	O	O	O	O	O	P	P	O	O	P
L2T	P	P	O	O	O	P	P	P	P	O	P	O	P	P	O	P	P	P	P	P	O	O	O
L4T	P	P	P	P	O	P	P	P	P	O	O	P	O	O	O	O	O	O	P	S	O	O	P
L4JS	P	P	P	P	P	P	P	P	P	O	O	P	O	O	O	O	O	O	P	P	O	O	P
L4JL	P	P	P	P	O	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P

*Results shown in table are those observed at final reading.
[1]Fermentation results apply to production of acidity.
P - designates positive reaction.
O - designates negative reaction.
S - designates slight positive reaction.

TABLE VI

PHYSICLOGICAL REACTIONS OF BACTERIA ISOLATED FROM SPOILED PICKLES*

									Fermentation Reactions[1]													
	Casein Hydrolysis	Gelatin Hydrolysis	Starch Hydrolysis	Nitrate Reduction	Urease Production	Citrate Utilization	Growth In pH 6.0	Voges-Proskauer	Fructose	Lactose	Sucrose	Maltose	Galactose	Raffinose	Arabinose	Xylose	Mannose	Mannitol	Glucose	Glycerol	Rhamnose	Inulin	Dextrin
W4	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W5	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W6b	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W6p	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W7	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W8	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W9	P	P	P	P	O	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W10	P	P	O	P	O	P	P	P	P	O	P	O	P	P	O	P	P	P	P	P	O	O	O
W11	P	P	P	P	O	P	P	P	P	O	O	P	O	O	O	O	O	O	P	P	O	O	P
W12	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
W13	P	P	P	P	P	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P
L1T	P	P	P	P	O	P	P	P	P	O	O	P	O	O	O	O	O	O	P	P	O	O	P
L2T	P	P	O	O	O	P	P	P	P	O	P	O	P	P	O	P	P	P	P	P	O	O	O
L4T	P	P	P	P	O	P	P	P	P	O	O	P	O	O	O	O	O	O	P	S	O	O	P
L4JS	P	P	P	P	P	P	P	P	P	O	O	P	O	O	O	O	O	O	P	P	O	O	P
L4JL	P	P	P	P	O	P	P	P	P	P	P	P	P	P	O	P	P	P	P	P	P	P	P

*Results shown in table are those observed at final reading.
[1]Fermentation results apply to production of acidity.
P - designates positive reaction.
O - designates negative reaction.
S - designates slight positive reaction.

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