FACTORS INFLUENCING THE FORMATION OF ROPY BRINE IN CUCUMBER FERMENTATION

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Introduction

Ropy or slimy fermentation is frequently encountered in certain industries. It occurs more commonly in the dairy and sugar industries. Investigators who have studied the cause of the ropiness have reported it as being due to certain bacteria. During the course of studies on cucumber fermentation, a great many samples of brine from both dill and salt pickles have been received for examination. The pickle packers from whom the samples were received were at a loss to understand the cause of the ropiness and asked for assistance in helping to determine the cause and, if possible, definite recommendations for its prevention. In view of these facts, a study was made to determine the cause of ropy brine and possible means for its control.

Review of the Literature

Stark and Foter (1931), investigating ropiness in milk, studied the reactions of bacteria isolated from various sources, and grouped them into seven classes; (1) A rod, gram negative, gas producing, and which curdles milk; (2) A rod, gram negative, gas producing, and which does not curdle milk. This bacterium

produces sliminess only in the presence of a sugar which is fermentable, (3) A coccus, gram positive, fermenting none of the sugars used, causing milk to have an alkaline reaction, and which, at 37°C., gives rise to organisms which appear to be rods, (4) A coccus, gram positive, digests milk at room temperature, at 45°C. produced an acid curd, and which reduces the litmus of litmus milk, (5) A Staphylococcus, gram positive, fermenting glucose, sucrose, and lactose, with no gas formation. This organism also will produce sliminess only when a sugar, which is fermentable, is present. (6) A Staphylococcus, gram positive, and which will ferment only glucose, (7) A coccus, gram positive, and no other noted characteristics.

Hammer (1928), is of the opinion that Bacterium viscosum and organisms of the Escherichia-Aerobacter group are the two principal causes of ropiness in milk in the United States. Bacterium viscosum has been isolated from ropy milk many times. The organism possesses a large capsule and produces a slow acid change in the milk. Adametz, who is thought to be the first one to isolate this organism, obtained it from water. Surface waters are thought to be an important source of this organism and difficulties with ropiness have been known to follow the flooding of pasture lands. Bacteria of the Escherichia-Aerobacter class have been isolated from

numerous cases in the United States. Ropiness in this instance also is due to capsule production, and acid production is not considered of paramount importance.

Ropiness has been observed in conjunction with acid production. The important members of this group are Streptococcus lactis, Lactobacillus casei, and Lactobacillus bulgaricus. With organisms producing lactic acid, ropiness can be observed when the acid reaction of the medium is still low. With an increase in acidity, there is a corresponding decrease in ropiness. The ropiness in this case is thought to be not so much due to the presence of capsules as to the relative numbers of organisms per cubic centimeter.

Buchanan and Hammer (1915) report the incidence of ropiness in cream when Bacterium visco-symbioticum was grown in the presence of certain strains of Streptococcus lactis. When grown together, a lower acidity was exhibited and a greater number of organisms resulted than from pure cultures of either organism. Again in this occurrence the ropy condition is explained by the large number of organisms present.

Organisms of the Leuconostoc group were reported as forming slime in syrups which were to be converted into sugar. Slime occurred in the syrups in large masses. These globular masses, upon microscopic

examination, proved to be clumps of organisms enclosed by individual capsules. Zettnow (1907), reported a characteristic slimy, opaque growth of Leuconostoc cultures inoculated into a sucrose gelatin medium. Beijerinck, according to Hucker and Pederson (1931), observed these same characteristics while studying Lactococcus dextranicus and reported that the slime was a dextran. Orla-Jensen, according to Hucker and Pederson (1931), found that some members of his Betacoccus group, isolated from slimy sugar solutions, produced a slimy growth in sucrose gelatin. Hucker and Pederson (1931) note that Leuconostoc obtained from vegetables produced slimy growth in two or three days under optimum conditions, especially those strains which were obtained from sauerkraut.

Pederson (1931) says: "Slimy or ropy kraut, although not common, may at times prove to be very annoying. Sliminess is ordinarily caused by certain strains of Lactobacillus cucumeris or Lactobacillus plantarum, the non-gas-producing rods. They grow very rapidly, especially when the temperature is raised, and become enveloped within a slimy material which causes the organisms to adhere to each other. The kraut may sometimes become normal after further curing."

Different organisms ferment glucose to a gummy substance called dextran. The dextran is one of the

substances found in the capsule surrounding the organism and is considered a secondary product of fermentation. Some of the organisms producing this viscous fermentation are Leuconostoc mesenteriodes and Bacterium pediculatum. Bacteria producing dextran have an optimum temperature range of from 30-35°C., and growth is best when no air is present. Dextrans are the cause of ropiness in wine and "frog-spawn" in sugar factories.

In sugar factories, a common observance is the conversion of sucrose into dextran. The organism studied most in connection with the formation of dextran is Leuconostoc mesenteroides.

Leuconostoc and other dextran producers first invert the sucrose into d-glucose and d-fructose, later forming the polysaccharide, dextran. Dextran is a part of the slimy capsule of the organism. Bacterium pediculatum (A. Koch and Hosaeus) acts in a manner analogous to the Leuconostoc in the syrup of sugar factories. This organism also possesses a slimy capsule.

Micrococcus gelatigenosus, Bacillus gummosus, Bacterium gummosum, and others produce dextran from sucrose and not from glucose. Most of these bacteria secrete invertase and it is thought that the sucrose in this instance also is first inverted. The action of

bacteria in forming gum from sucrose and not from glucose is explained by the fact that the glucose can only be fermented in its nascent state.

The gum produced by organisms during viscous fermentation is not always dextran. Levulan may also form the slimy product.

Method

Samples of ropy or slimy brine were plated on yeast extract agar and incubated for forty-eight hours at 37°C. At the end of this time, representative colonies were fished from the plates to yeast extract agar slants. These cultures were purified by repeated platings on yeast extract agar and typical colonies transferred to slants of the same medium. Two sets of cultures representing a total of thirteen different organisms were isolated in this manner from four samples of ropy brine received at different times from Michigan and Wisconsin. The analyses of the samples are given in table 4. The morphological, cultural, and physiological characteristics of these organisms were studied in detail.

In order to determine the chemical and physical factors which were responsible for, or which induced the ropy condition found in the cucumber brines, the

following experiments were conducted. The first group of organisms isolated was subjected to varying concentrations of salt ranging from 0 per cent to 15 per cent. Triplicate sets of nutrient broth containing 0, 2.5, 5, 7.5, 10, 12.5 and 15 per cent NaCl were made and inoculated with cultures I, II, III, IV, V, VI, and VII. They were then incubated at temperatures of 15, 20 and 37°C. (See table 1.) The same group of organisms was then inoculated into a 5 per cent solution of plain broth adjusted to pH values ranging from 1-12, and incubated at a temperature of 37°C. (See table 2.)

For the second group of organisms, a slightly different procedure was followed. Instead of nutrient broth, pickle brine was used. Triplicate sets of brine were made so that it contained 5.3, 7.95, 10.6, and 13.25 per cent NaCl which corresponds to 20, 30, 40 and 50° salometer respectively. Each salt concentration was adjusted to a pH of 4, 5, 6, 7, and 8. Each set of brine was then inoculated with the different organisms and incubated at temperatures of 7, 20 and 37°C. respectively. (See table 3.)

Results

The cultures isolated from the various brines may be divided into two different groups on the basis

of their fermentation reactions. The organisms placed in Group I were encapsulated rods which did not ferment any of the carbohydrates tested. They include cultures I to VII inclusive. They grew in all concentrations of NaCl tested and at the three temperatures at which they were incubated. At temperatures of 20 and 37°C. all cultures, except I and VII, produced ropiness in the broth in all salt concentrations. At a temperature of 15°C. all cultures, except VII, produced ropiness in the broth in all salt concentrations. (See table 1.)

When the same cultures were inoculated in nutrient broth containing five per cent NaCl which was adjusted to pH values ranging from 1 to 12, ropiness did not appear in any broth below pH 5. At this low pH a slight amount of ropiness was produced by all the cultures. Cultures II and III produced more than the others. Ropiness was most abundant from pH 6 to 9. At pH's beyond 9 ropiness was reduced or absent due to the inability of the organisms to grow at these high pH's. (See table 2.)

The organisms in Group II were rods which fermented many of the carbohydrates tested. (See table 3.) They comprise cultures VIII to XIII inclusive. At a temperature of 7°C., none of the cultures, except VIII and XIII -S produced any ropiness below a pH of 5.

Cultures VIII and XIII-S produced ropiness at a pH of 4 but only when the percentage of NaCl was below 10.6 (40° salometer.) No ropiness was evident when a high acidity was used in combination with a high salt concentration.

When the incubation temperature was raised to 20°C., culture X was the only one that produced ropiness at a pH of 4. However, when the salt concentration was raised to 13.25 per cent (50° salometer) culture X no longer produced ropiness. Cultures VIII, IX and X showed abundant ropiness at 20°C. at pH 5, 6, 7 and 8 when the salt concentration was relatively low, 5.3 per cent (20° salometer).

No culture produced ropiness at 37°C. in a brine adjusted to pH 4 with the exception of culture X. This organism produced ropiness in brine at pH 4 containing 7.95 per cent (30° salometer) NaCl, but an increase of NaCl to 10.6 per cent (40° salometer) eliminated this condition.

Table 1 showing influence of temperature and salinity on ropiness in cucumber brine

Per cent NaCl	Temperature of Incubation																								
	15°C							20°C							37°C										
	Culture							Culture							Culture										
	I	II	III	IV	V	VI	VII	I	II	III	IV	I	VI	VII	I	II	III	IV	V	VI	VII			
0	+	-	±	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-				
2.5	±	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-				
5.	+	+	+	+	+	+	-	±	+	+	+	+	+	-	-	+	+	+	+	+	-				
7.5	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-				
10.	+	±	+	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-				
12.5	+	0	+	+	+	+	-	±	+	+	+	+	+	0	-	+	+	+	+	+	-				

+ ropiness - no ropiness ± doubtful ropiness 0 no growth

Table 2 showing influence of pH on ropiness in a five per cent brine at 37°C.

Culture	pH											
	1	2	3	4	5	6	7	8	9	10	11	12
I	0	0	0	0	±	±	±	±	±	0	0	0
II	0	0	0	0	+	+	++	++	++	+	0	0
III	0	0	0	0	+	+	+	+	+	+	0	0
IV	0	0	0	0	±	+	+++	+	+	+	0	0
V	0	0	0	0	±	+	+	+	+	0	0	0
VI	0	0	0	0	±	+	+	+	+	+	0	0
VII	0	0	0	±	±	+	+	±	0	0	0	0

0 no ropiness

± doubtful ropiness

\+ perceptible ropiness

++ moderate ropiness

+++ fairly abundant ropiness

Table 3. Showing influence of temperature, acidity and salinity on the production of ropiness in cucumber brine.

Culture VIII

pH	Temperature of Incubation											
	7°C.				20°C.				37°C.			
	Salinity-Percent salt				Salinity-Percent salt				Salinity-Percent salt			
	5.3	7.95	10.6	13.25	5.3	7.95	10.6	13.25	5.3	7.95	10.6	13.25
4	+	+	-	-	-	-	-	-	-	-	-	-
5	+	+	-	-	++++	-	-	-	++++	+	-	-
6	+	+	+	-	++++	+	+	-	++++	+	+	-
7	+	+	+	-	++++	+	++	-	++++	+	+	-
8	-	+	+	+	++++	+	+	-	++++	++	+	-
	Culture IX											
4	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	-	-	++++	+	-	-	++++	+	-	-
6	+	+	+	-	++++	+	+	-	+++	++	+	-
7	+	++	+	+	++++	+	+	+	++++	+	++	-
8	+	+	+	-	++++	+	+	+	++++	+	++	-

Table 3 Continued

					Culture X							
4	-	-	-	-	++	++	+	-	-	++	-	-
5	+	-	-	-	++	++	+	+	++++	++	-	-
6	+	+	+	+	++	+	+	+	++	+	+	-
7	+	+	+	+	++	++	+	-	+++	+	+	-
8	+	-	+	+	++	+	++	+	++	++	+	-
					Culture XI							
4	-	-	-	-	-	-	-	-	-	-	-	-
5	+	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	++	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
					Culture XII-S							
4	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	-	-	+	-	-	-	++	-	-	-
6	-	-	-	-	++	+	-	-	++	-	-	-
7	-	+	+	-	+	++	-	-	-	-	-	-
8	+	+	+	-	-	+	-	-	+	+	-	-

Table 3 Continued

					Culture XII-R							
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
					Culture XIII-S							
4	+	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	++	-	-	-	+++	-	-	-
6	-	-	-	-	+	++	-	-	+	-	-	-
7	-	-	-	-	-	+	-	-	-	-	-	-
8	-	-	-	-	-	+	-	-	-	-	-	-

Table 3 Continued

	Culture XIII-R											
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	++	-	-	-	++	-	-	-
6	-	-	-	-	+	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-

- No ropiness + Perceptible ropiness ++ Moderate ropiness
+++ Fairly abundant ropiness ++++ Abundant ropiness

5.3 Per cent - 20° salometer

7.95 Per cent - 30° salometer

10.6 Per cent - 40° salometer

13.25 Per cent - 50° salometer

S - smooth form of organism

R - rough form of organism

Table 4 showing analysis of samples of ropy brine from which bacteria were isolated.

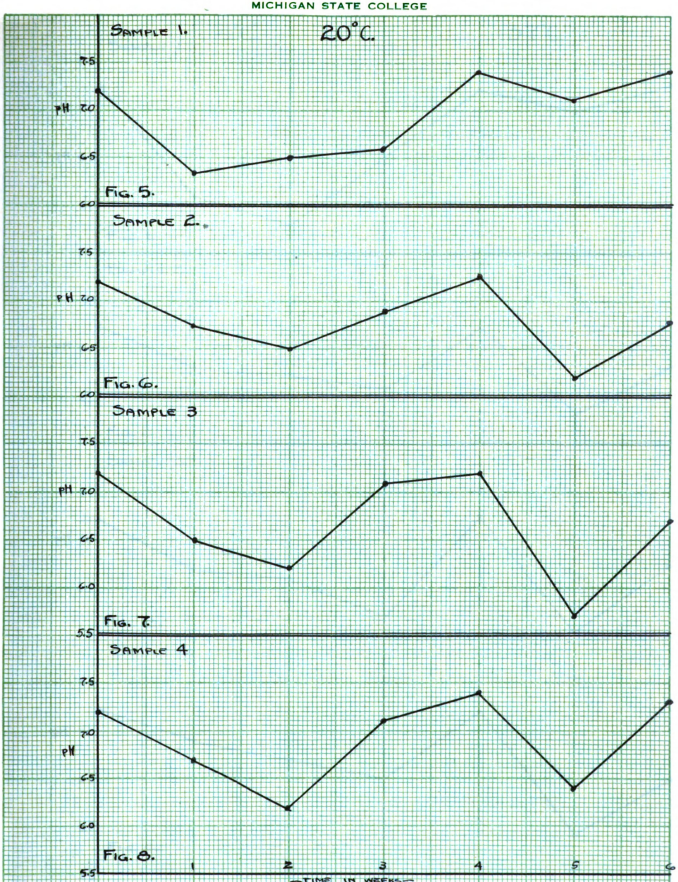
Sample No.	pH	Per Cent Acetic Acid	Per Cent Lactic Acid	Salometer
1	4.3	0.7	1.1	Not Sufficient
2	5.8	0.1	0.025	Not Sufficient
3	4.7	0.3	0.4	18
4	5.4	0.2	0.2	16

Influence of New Brine on Ropiness

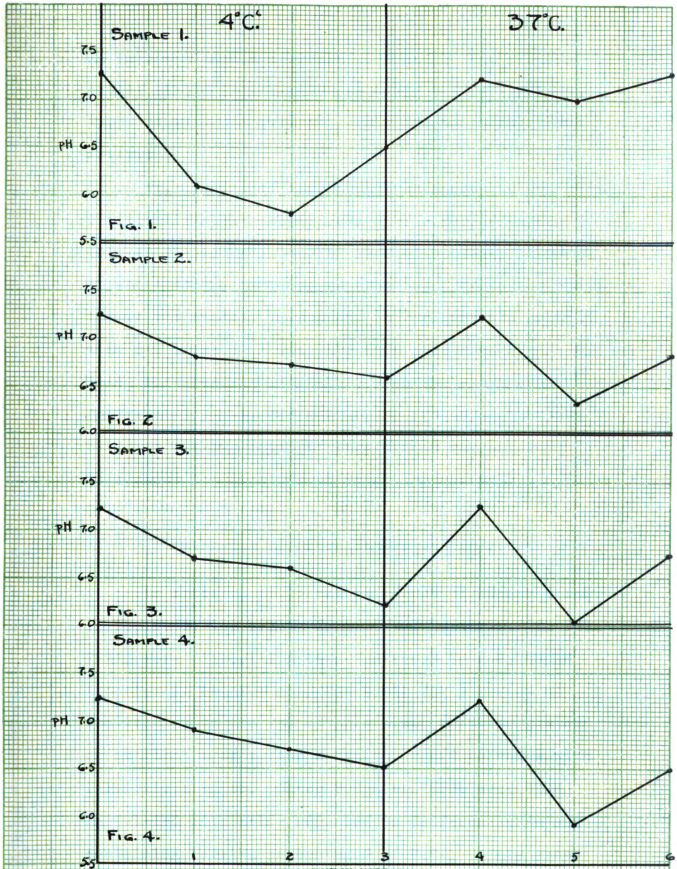
In order to study the influence of new brine on ropiness under more nearly natural conditions, six cubic centimeters of brine from each sample of ropy brine were inoculated into 100 c.c. of 5.3 per cent (20° salometer) brine. The inoculated flasks were then placed at temperatures of 4, 20 and 37°C. (See Figures 1 to 8). One set of flasks was incubated at 4°C. for three weeks and then incubated at 37°C. for three more weeks. A duplicate set of inoculated flasks was incubated at 20°C. for six weeks. The pH of both sets was adjusted to 7.2. The pH of the flasks was determined each week.

When the flasks were incubated at 4°C., the pH of the brine slowly decreased at this temperature indicating that there was a growth of acid producing bacteria. However, when the flasks were incubated at 37°C., there was an increase in pH for the first week, after which time there was a decrease and then an increase again of the pH. The interesting thing is that ropiness did not appear at either the low or high temperatures.

At a temperature of 20°C., the pH of the brine followed the same general trend as for the low and high temperatures. There was a decrease followed by



Figs. 5, 6, 7, & 8. Graphs showing the pH values of a 5.3 per cent (20° Salometer) brine when inoculated with ropy brine and incubated at 20°C. for six weeks.



Figs 1, 2, 3 & 4 - Graphs showing the influence of teperature on the acidity of a 5.3 Per cent (20° Salometer) brine when inoculated with ropy brine.

an increase then a decrease of the pH. The pH of course simply reflects the activity of the different types of bacteria present. At 20°C. no ropiness was observed.

This series of experiments explains why ropiness practically always disappears when a pickle packer changes the brine on a ropy tank of pickles. It would appear that the bacteria causing ropiness do not adapt themselves readily to changes in environment; so that when new brine is added, the acid producing bacteria, being more readily adaptable, gain the ascendency. The fact that ropiness did not appear indicates that the bacteria causing ropiness remained in the minority throughout the duration of these experiments.

Influence of Dissociation on Ropiness

In order to study the influence of dissociation on ropiness, the smooth form of two cultures, XII and XIII, were dissociated into the rough form by rapid transfer in lithium chloride broth for a period of two weeks. At the end of this time, cultures plated on nutrient agar produced typical rough colonies with filamentous edges. These rough cultures were then inoculated into brine adjusted to different pH's and containing varying percentages of NaCl. They were

incubated at temperatures of 7, 20, and 37°C. (See table 3).

A comparison of the smooth and rough cultures of XII and XIII incubated in brine under the same conditions, shows that the smooth form of the cultures produced ropiness much more readily than did the rough cultures. In fact culture XII-R, i.e. the rough form of culture XII, produced no ropiness in any of the brines while the smooth form of XII produced ropiness at the lower salt concentrations. In the case of culture XIII, there was not such a marked difference between the smooth and rough form of the cultures, but the smooth form produced more ropiness, than the rough form.

The rough forms of the cultures were not very stable and readily reverted to the smooth form. It is evident that dissociation does not play an important part in producing ropiness in cucumber brine.

Description of Cultures

I

Rods: 0.7-1 Micron. Motile. Gram-negative.
Uncapsulated. No spores.

Gelatin stab: No liquefaction.

Agar colonies: Smooth, entire, indistinct.

Agar slant: Slight, white, filiform.

Broth: No growth.

Litmus milk: Unchanged.

Potato: No growth.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch not hydrolyzed.

Ammonia not formed.

Yeast extract agar slant: Dull, slight growth,
smooth, grayish.

H_2S not formed.

II

Rods: 0.5-0.7 by 1 microns. Motile. Gram-negative. Encapsulated. No spores.

Gelatin stab: Saccate liquefaction.

Agar colonies: Smooth, entire, slightly raised.

Agar slant: Moderate, grayish, glistening, slimy, filiform.

Broth: Pellicle with no sediment.

Litmus milk: Rennet curd, peptonization, reduction of litmus, and alkaline in reaction.

Potato: Spreading, grayish, thick.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Glistening, luxuriant, slimy, and grayish.

H_2S not formed.

III

Rods: 0.7 by 1 microns. Motile. Gram-negative. Encapsulated. No spores.

Gelatin stab: Crateriform to stratiform liquefaction.

Agar colonies: Smooth, lobate, convex.

Agar slant: Moderate, grayish, filiform, glistening, slimy.

Broth: Pellicle with no sediment.

Litmus milk: Rennet curd, peptonization, reduction of litmus, and alkaline in reaction.

Potato: Spreading, yellow to gray, slightly slimy.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Glistening, luxuriant, grayish, slimy.

H_2S not formed.

IV

Rods: 0.5 by 1 microns. Motile. Gram-negative. Encapsulated. No spores.

Gelatin stab: Stratiform liquefaction.

Agar colonies: Smooth, entire, convex.

Agar slant: Moderate, grayish, filiform, glistening, slimy.

Broth: Pellicle with a viscid sediment.

Litmus milk: Reduction of litmus, peptonization, and alkaline in reaction.

Potato: Spreading, cream colored, slightly slimy.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Glistening, luxuriant, slimy, grayish.

H_2S not formed.

V

Rods: 0.5 by 1 microns. Motile. Gram-negative. Encapsulated. No spores.

Gelatin stab: Stratiform to saccate liquefaction.

Agar colonies: Smooth, entire, convex.

Agar slant: Moderate, gray to buff color, glistening, raised, filiform, undulate border, slimy.

Broth: Pellicle with no sediment.

Litmus milk: Rennet curd, peptonization, reduction of litmus, and alkaline in reaction.

Potato: Spreading, yellow to gray, slightly slimy.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Glistening, slimy, luxuriant, grayish.

H_2S not formed.

VI

Rods: 0.7 by 1 microns. Motile. Gram-negative. Encapsulated. No spores.

Gelatin stab: Stratiform liquefaction.

Agar colonies: Smooth, entire, convex.

Agar slant: Moderate, grayish, glistening, filiform, slimy.

Broth: No pellicle, viscid sediment.

Litmus milk: Reduction of litmus, rennet curd, peptonization, and alkaline in reaction.

Potato: Filiform, cream colored, slightly slimy.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Glistening, slimy, luxuriant, grayish.

H_2S not formed.

VII

Rods: 1.0 by 2.1 microns. Non-motile. Gram-negative. Encapsulated. No spores.

Gelatin stab. Stratiform to saccate liquefaction.

Agar colonies: Rough, curled, slightly raised.

Agar slant: Abundant, white, spreading, glistening, echinulate, butyrous.

Broth: No pellicle, flaky sediment.

Litmus milk: Rennet curd, reduction of litmus, and slightly acid in reaction.

Potato: Gray, spreading, thick, slimy.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Glistening, grayish, slight growth.

H_2S not formed.

VIII

Rods: 0.5 by 1.5 microns. Motile. Gram-negative. Encapsulated. No spores.

Gelatin stab: Stratiform liquefaction.

Agar colonies: Smooth, slightly notched, slightly raised.

Agar slant: Moderate, grayish, glistening, echinulate, butyrous, slightly rugose.

Broth: Pellicle, viscid sediment.

Litmus milk: Rennet curd, reduction of litmus, peptonization and acid in reaction.

Potato: Scanty, brown to grey, filiform.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch not hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Rugose-membranous, dull, luxuriant, grayish.

H_2S not formed.

IX

Rods: 0.7 by 2.1 microns. Motile. Gram-negative. Encapsulated. No spores.

Gelatin stab: Stratiform liquefaction.

Agar colonies: Smooth, lobate, convex.

Agar slant: Moderate, grayish, glistening, slightly rugose, echinulate, butyrous.

Broth: Pellicle, no sediment.

Litmus milk: Reduction of litmus, peptonization, rennet curd, and acid in reaction.

Potato: Scanty, brown, filiform.

Indol not formed.

Nitrates slightly reduced to nitrites.

Sugar reactions: See table 5.

Starch not hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Rugose-membranous, dull, luxuriant, grayish.

H_2S not formed.

X

Rods: 0.5 by 1.5 microns. Motile. Gram-negative. Encapsulated. No spores.

Gelatin stab: Stratiform liquefaction.

Agar colonies: Smooth, entire, convex.

Agar slant: Moderate, grayish, filiform, glistening, butyrous.

Broth: Pellicle, granular sediment.

Litmus milk: Reduction of litmus, rennet curd, peptonization, and acid in reaction.

Potato: Scanty, yellow to brown, filiform.

Indol not formed.

Nitrates slightly reduced to nitrites.

Sugar reactions: See table 5.

Starch not hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Rugose-membranous, dull, luxuriant, grayish.

H_2S not formed.

XI

Rods: 0.7 by 1.5 microns. Motile. Gram-negative.
No capsule. No spores.

Gelatin stab: Saccate liquefaction.

Agar colonies: Smooth and raised.

Agar slant: Moderate, grayish, filiform, glistening, butyrous, slightly rugose.

Broth: Pellicle, slight flaky sediment.

Litmus milk: Rennet curd, peptonization, reduction of litmus, and neutral in reaction.

Potato: Scanty, brown to gray, filiform, butyrous.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch not hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Rugose-membranous, dull, luxuriant, grayish.

H_2S not formed.

XII

Rods: 1.0 by 2.0-2.5 microns. Motile. Gram-negative. No capsule. No spores.

Gelatin stab: Saccate liquefaction.

Agar colonies: Rough, curled, flat.

Agar slant: Abundant, cretaceous, glistening, spreading, butyrous.

Broth: Pellicle, flaky sediment.

Litmus milk: Rennet curd, peptonization, reduction of litmus, and alkaline in reaction.

Potato: Abundant, spreading, cream colored, thick, butyrous.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Dull, luxuriant, dirty gray, butyrous.

H_2S not formed.

XIII

Rods: 1.0 by 2.0-2.5 microns. Motile. Gram-negative. No capsule. No spores.

Gelatin stab: Saccate liquefaction.

Agar colonies: Rough, curled, flat.

Agar slant: Abundant, cretaceous, glistening, spreading, butyrous.

Broth: Pellicle, flaky sediment.

Litmus milk: Rennet curd, peptonization, reduction of litmus and alkaline in reaction.

Potato: Abundant, spreading, cream colored, thick, butyrous.

Indol not formed.

Nitrates reduced to nitrites.

Sugar reactions: See table 5.

Starch not hydrolyzed.

Ammonia formed.

Yeast extract agar slant: Dull, luxuriant, dirty gray, butyrous.

H_2S not formed.

Table 5 showing fermentation reactions of the organisms isolated from ropy brine.

Culture	Lactose	Mannit	Sucrose	Maltose	Dextrose	Raffinose	Galactose	Levulose	Arabinose	Dextrin	Glycerol
I	-	-	-	-	-	-	-	-	-	-	-
II	-	-	-	-	-	-	-	-	-	-	-
III	-	-	-	-	-	-	-	-	-	-	-
IV	-	-	-	-	-	-	-	-	-	-	-
V	-	-	-	-	-	-	-	-	-	-	-
VI	-	-	-	-	-	-	-	-	-	-	-
VII	-	-	-	-	-	-	-	-	-	-	-
VIII	+	+	+	+	+	±	-	+	+	+	-
IX	-	±	+	+	+	±	+	+	+	-	-
X	-	±	+	+	+	+	+	+	-	-	-
XI	-	-	-	-	+	-	-	+	-	-	-
XII-S	-	-	+	+	+	-	-	+	-	+	-
XII-R	-	-	+	+	+	-	-	+	-	+	-
XIII-S	-	-	+	+	+	-	-	+	-	+	-
XIII-R	-	-	+	+	+	-	-	+	-	+	-

+ acid - no reaction ± doubtful reaction

S - Smooth form of the organism

R - Rough form of the organism

Classification of Organisms

The identification of the organisms isolated presented an exceedingly complex problem. Variations in cultural characteristics prevented any definite conclusions.

Group I, comprising cultures which did not ferment any of the carbohydrates tested, offered many possibilities. The genus Achromobacter most nearly coincided with characteristics obtained from the cultures used in this experiment. It was thought that some plant pathogen might be responsible for the ropiness, particularly the genus Phytomonas. Similarities were noted but nothing that warranted placing them in this group. One possibility remains and that is the organisms isolated were involution forms which do not conform to the characteristics, usually associated with this group.

Group II offered numerous variations such as to make identification impossible. Here again the possibility remains that involution forms are being studied. If such, it may be that the organisms belong to the genus Leuconostoc. The Leuconostoc, normally of coccus shape, are known to exist as involutionary rods.

It is also known that there exists in soil a large group of bacteria having many characteristics, common to the plant pathogens but which are not plant pathogens. It is most likely that this group of bacteria fall into this classification.

Discussion

It is apparent from this study that there are two distinct groups of bacteria which are capable of causing ropiness in cucumber brine. Group I comprising cultures I to VII inclusive are short, encapsulated, gram negative, motile (culture VII nonmotile) rods which do not ferment any of the carbohydrates tested; and Group II comprising cultures VIII to XIII which are medium sized, gram negative, motile rods. Cultures VIII, IX and X of this group are encapsulated while cultures XI, XII and XIII have no capsules under ordinary conditions of growth. The members of Group II ferment many of the carbohydrates tested.

Despite the morphological, cultural, and physiological differences between the two groups and between the different cultures in the same group it is obvious that when certain physical and chemical conditions are present in the brine, ropiness occurs. The three conditions which have been studied are acidity, salinity, and temperature. It is apparent from the results obtained that ropiness does not occur in a brine with a real high or real low pH. The minimum pH is 6 and the maximum 9 in the majority of cases, while the optimum is between 7 and 8. The significant fact is that ropiness occurred in brine with a low acid content.

The influence of salinity on ropiness is also evident from these experiments. Ropiness occurs most frequently in all the cultures at the low salt concentrations. As the salt concentration is increased, ropiness disappears. For example in brine containing 13.25 per cent salt (50° salometer) there are only a very few instances when ropiness is found. It should also be noted that where it does occur at this salinity, the pH is always high, i. e. there is no acid present. Fabian, Bryan, and Etchells (1932) have called attention to the relationship between high acidity and salinity and its value in suppressing undesirable types of bacteria during fermentation of cucumbers. They had in mind the suppression of bacteria causing softening of the cucumbers during curing. However, it has been shown in these experiments that it is equally valuable in controlling ropiness.

Whenever ropiness appears in a tank of fermenting pickles, many pickle packers immediately draw off the brine and replace it with fresh brine. The experiments reported here show that when ropy brine is diluted with fresh brine that the ropiness did not reappear. However, they also show that there is a better way of getting rid of the ropiness than this, and that is by increasing the salt concentration. If when the brine on a tank of

pickles becomes ropy, the salt concentration is increased at a more rapid rate than normal then the ropiness will disappear inside of two or three days. This would be better than drawing off the ropy brine and replacing it with new brine since this is expensive and troublesome; but what is still more important the discarded brine contains considerable food materials which are important for a successful fermentation of the cucumbers.

The influence of temperature on ropiness is also evident. Ropiness is more common at the higher than at the lower temperatures. The bacteria causing ropiness grow better at higher than at lower temperatures. This would indicate that during the cucumber season if the weather should be exceptionally hot, we might expect to experience more trouble with ropy brine than in normal weather.

The conditions which would be highly favorable for ropy brine in a tank of pickles would be when the cucumbers were first put in a tank before active fermentation had started. If during this time the weather were hot, the three principal factors responsible for ropiness would be present, viz., low acidity, low salinity, and a high temperature. There are doubtless other conditions which are favorable and other factors which are responsible for ropy brine but these three appear outstanding.

Summary and Conclusions

Thirteen cultures of bacteria were isolated from four samples of ropy brine received from Michigan and Wisconsin. They were divided into two different groups on the basis of their morphological, cultural, and physiological characteristics.

Group I, comprising seven cultures, consisted of bacteria which were short, motile (except culture VII which was non-motile), gram negative, encapsulated rods which did not ferment any of the carbohydrates tested. Group II, comprising six cultures, consisted of bacteria which were motile, gram negative rods somewhat longer than those of Group I. Three members of this group were encapsulated.

A study was made of the optimum conditions for the production of ropiness when these thirteen cultures were inoculated into cucumber brine. The three conditions studied were acidity, salinity, and temperature. It was found that the cultures produced the greatest amount of ropiness in a brine having a low acidity and a low salinity. The amount of ropiness likewise increased with a rise in temperature.

The influence of dissociation on ropiness was studied. The rough form of the two organisms studied produced little or no ropiness compared to the smooth form.

A study was made of the influence of temperature on the production of ropiness in brine when inoculated with ropy brine containing a mixed culture of bacteria.

This study elucidates many points which help to explain the cause of ropy brine frequently encountered in industry and should be of value in controlling this condition.

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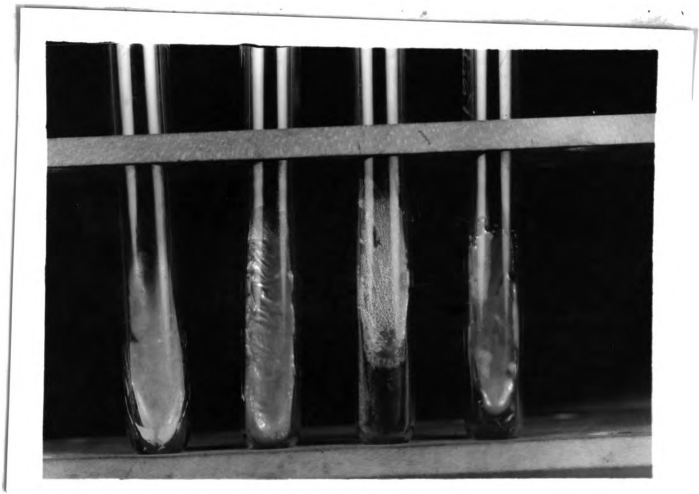

Figure 9. Photograph of agar slant cultures of organisms isolated showing type of growth exhibited by cultures.

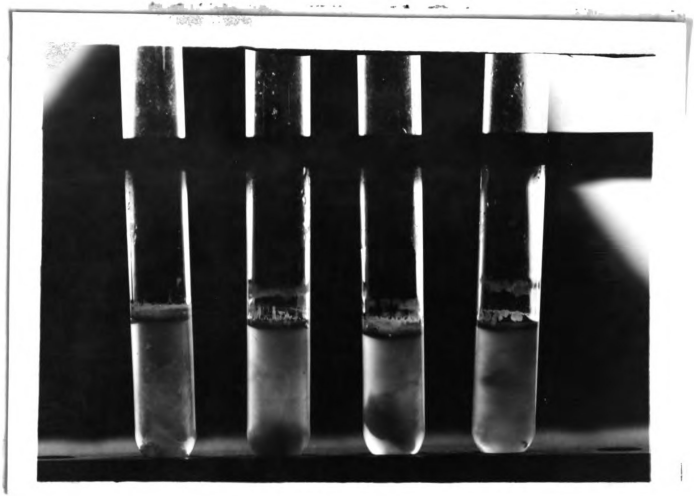

Figure 10. Photograph showing abundant production of ropiness in brines.

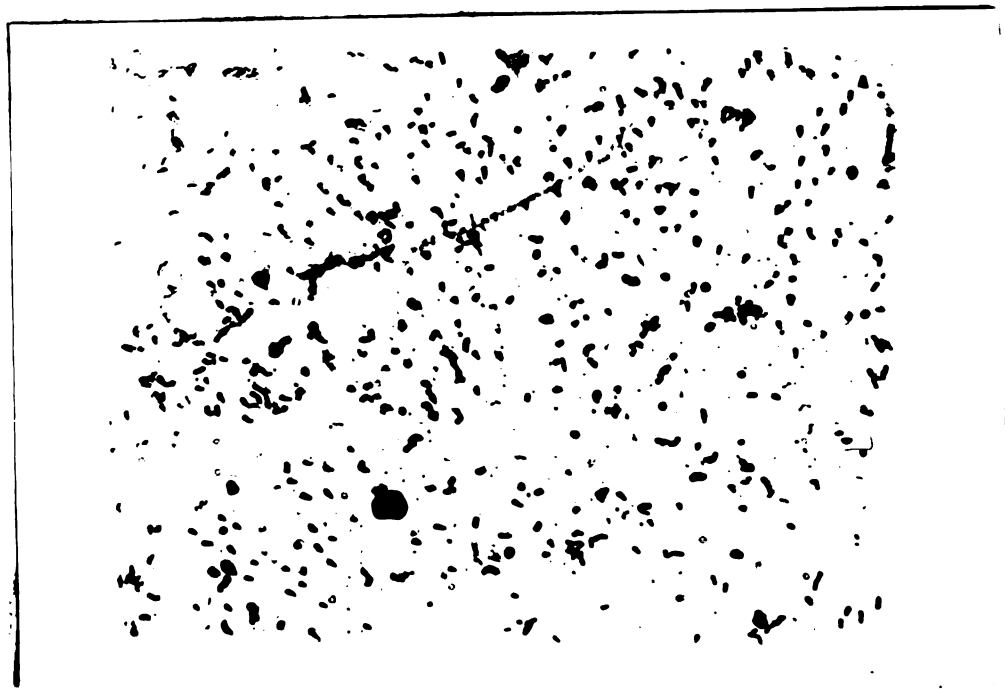

Figure 11. Photomicrograph showing encapsulated forms of organisms isolated. 450 X

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