LEUCONOSTOC MESENTEROIDES AS A CAUSE OF ROPINESS IN CANNED PEACHES

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LEUCONOSTOC MESENTEROIDES AS A CAUSE OF ROPINESS IN CANNED PEACHES

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Introduction

Ropy fermentation is often encountered in the different food industries. It occurs most often in the dairy and sugar industries. The cause of ropiness, as determined by investigators, has been bacterial in origin.

In the fall of 1949, the Michigan Agricultural Laboratory, Lansing, Michigan, submitted samples of canned peaches in which the syrup had become ropy. Bacteriological analysis was made of the samples and representative colonies were picked from the plates which were purified and identified according to the methods outlined in "The Manual of Methods for Pure Culture Study of Bacteria".

Literature Review

Ehrenberg (1840) was probably the first to observe specific organisms in ropy milk. Since that time over forty different microorganisms have been shown to cause ropiness in milk and milk products.

Buchanan and Hammer (1915) in a scholarly and comprehensive paper presented a key to thirty-three organisms implicated in ropy milk outbreaks. Hammer and Cordes (1920) reported Micrococcus cremoris-viscosi as the causative organism in a ropy milk outbreak.

Stark and Foter (1931) grouped ropy milk organisms into the following groups: (1) A gram negative rod, which produced gas, and curdled milk. (2) A gram negative rod, which produced gas, but did not curdle milk and produced ropiness only in the presence of a fermentable sugar. (3) Gram positive cocci, which fermented none of the sugars used, caused the milk to have an alkaline reaction, and at 37°C gave rise to organisms which appeared to be rods. (4) Gram positive cocci which digested milk at room temperature, and at 45 °C produced an acid curd which reduced litmus milk. (5) A gram positive staphylococcus which fermented glucose, sucrose, and lactose, with no gas formation, and produced ropiness only when a fermentable sugar was present. (6) A gram positive staphylococcus which fermented only glucose. (7) A gram positive coccus, with no other noted characteristics.

Sarles and Hammer (1933) found that ropiness in dairy products may also be caused by; Escherichia neapolitana, Aerobacter aerogenes, and Aerobacter cloacae.

Davis (1935) reported that Pseudomonas and Corynebacteria may cause ropiness in milk.

Three strains of Alcaligenes viscosus produced pronounced ropiness in milk at 18°C, 25°C, and 30°C, but failed to produce ropiness in milk which became acid due to action of lactic acid bacteria present; according to Beck and Chase (1938).

Rodenkirchen (1939) described a transitional form between a micrococcus and a sarcina (apparently Sarcina auratica) which caused ropiness in cream.

Ropiness is often associated with the production of acid. Streptococcus lactis, Lactobacillus casei, and Lactobacillus bulgaricus, show ropiness which is evident before much acid is produced.

Micrococcus or Achromobacter were isolated from ropy chocolate milk by Tarnutzer (1939). These organisms would not produce a ropy condition in regular milk. Apparently they required carbohydrates other than lactose.

An outbreak of ropiness in commercial pasteurized milk was studied by Prouty (1943) and a thermoduric micrococcus closely related to Micrococcus freudenreichii was isolated. This organism survived a pasteurization temperature of 143°F for 20 to 35 minutes.

In Great Britain a similar organism was isolated from new hay by M'Kenzie, Morgan, and Parker (1943).

Ropiness in sauerkraut according to Pederson (1931) is ordinarily caused by certain strains of Lactobacillus cucumeris or Lactobacillus plantarum. These organisms grew rapidly and became enveloped in a slimy material which caused the organisms to adhere to each other. The kraut may become normal after further curing.

Ropy cider was studied by Kayser (1911) who was able to isolate four different organisms. However he

felt that the ropiness might be due to factors other than the presence of bacteria.

Edson (1910) isolated a new species Bacillus aceris as a causative organism of ropy sap. Later Edson, Jones, and Carpenter (1912) reported strains of Pseudomonas flourescens which caused ropiness in maple sap. They also found a new species named Bacillus parallelus causing ropiness in sap.

Fabian and Buskirk (1935) isolated A. aerogenes as a causative organism of ropiness in maple syrup.

Fabian and Nienhuis (1934) working with ropy brine in cucumber fermentation isolated an organism which closely coincided with the characteristics of the genus Achromobacter. Another group of organisms isolated from ropy brine were thought to be involution forms of Leuconostoc.

A case of ropiness in tea was investigated by Greene, Judd, and Marx (1940). They found that the ropiness was caused by A.aerogenes in the water supply.

Schroder (1930) described ropiness in sausage which he found was due to cocci which grew best on blood and serum agar but was nonpathogenic to men and white mice.

The first to isolate the organism causing ropiness in bread was probably Laurent (1885), in Belgium. He named the causal organism Bacillus panificans. In Vienna Kratschmer and Niemilowicz (1889) showed that

Bacillus mesentericus vulgatus was the organism that caused ropy bread. The two organisms are probably the same. In the United States Russell (1898) reported B. mesentericus vulgatus as the causal organism. Workers generally agreed that B. mesentericus vulgatus is the organism responsible for ropy bread.

Ropy meat curing solutions are caused by encapsulated bacteria. Heideman (1923) isolated five organisms capable of causing rope in curing solutions. Another organism causing ropiness in curing solutions was named Micrococcus lipolyticus by Horowitz-Vlasova, and Livshitz (1931). It closely resembled Micrococcus albus and Micrococcus flavusliquefaciens.

The development of ropiness in sugar solutions and in fruits possessing a large amount of sugar in the juice is not uncommon. Ropiness as we have seen may be caused by a large number of different organisms; one of which is the genus Leuconostoc.

The genus Leuconostoc is made up of but a few species. The great majority of which live on plant and vegetable materials. Leuconostoc mesenteroides has been isolated from sugar beets, sugar cane, milk and milk products, by Hucker and Pederson (1930). Vaughan, Douglas, and Gililland (1943) reported on its role in fermenting olives. They found that L. mesenteroides was the most prevalent of the lactic acid organisms present during the first stage of

fermentation of Spanish Type olives. During the second or intermediate stage it (L. mesenteroides) gained the ascendency over all types of organisms; however it disappeared in the early part of the third stage when L. plantarum predominated.

L. mesenteroides has been reported by Pederson (1930) to be largely responsible for the early production of acid in the fermentation of sauerkraut. Hohl (1942) found it present in the early stages of lettuce fermentation, but it disappeared after the sixth day. Hartsell (1944) was able to isolate it from spray dried egg powder. L. mesenteroides has also been reported by Spiegelberg (1940) to be the cause of swells in canned fruit.

L. mesenteroides may be cultivated on nutrient beet or cane sugar juices in which, at favorable temperatures, it will decompose large quantities of sugar with the resulting production of a slime or ropy condition. In sugar factories this condition is known as "frog-spawn"; which inhibits crystallization of the sugar from the liquor. Abundant growth of the slime may block the pipes and fittings of the sugar factories.

Kircher (1839) and Feltz (1874) both observed microorganisms which were probably Leuconostoc in slimy beet juice. The "frog-spawn" phenomenon led Scheibler (1869) to study slimy beet juice in an effort

to determine its cause. He believed that the slime was formed from the protoplasm of the beet cell by enzymic action.

Jubert (1874) did not agree with Scheibler's work and showed that the power of slime formation was destroyed by heating to 90°C (194°F) or treating with phenol, thus indicating the presence of a microorganism. Scheibler in further studies isolated a gum-like substance which he called dextran.

Cienkowski (1878), was the first to study the slime formation from a microbiological approach, isolated a species he called Ascococcus mesenteroides as the cause of "frog-spawn".

Van Teighem (1878) determined the size of Cienkowski's organism and observed the endospore formation. The organism reminded him of the blue-green algae genus Nostoc so he named the bacterium genus Leuconostoc or white Nostoc.

Liesenberg and Zopf (1892) were the first to isolate strains of L. mesenteroides in pure culture form, and succeeded in purifying them by virtue of their resistance to heat. After Liesenberg and Zopf a number of workers isolated and studied strains of Leuconostoc. There was much duplication of effort and different names were given to the same species.

It was not until Hucker and Pederson (1930) in an excellent paper on the cultural characteristics and

taxonomic relationships of the Leuconostoc was order established. They isolated Leuconostoc from slimy sugar solutions, fermenting vegetables, and from milk and milk products. They considered the Leuconostoc as a morphological intermediate between the Streptococci and Lactobacilli. Under certain conditions the cells were elongated, while in slowly growing cultures the spherical form predominated. In ordinary yeast extract broth they grew as cocci and sometimes formed chains. In acid sauerkraut juice or in acid fruit juices they often grew as rods 0.5 to 1.0 microns in length.

Hucker and Pederson (1930) also found that the different species within the genus could be differentiated on the basis of sucrose fermentation. Those species which did not ferment sucrose were usually found in milk and milk products. Those species fermenting sucrose were further divided into two groups on the basis of pentose fermentation.

a. Those fermenting sucrose and pentose (xylose and/or arabinose).

b. Those that fermented sucrose but not pentoses.

The by-products of the carbohydrate fermentation were found to be; levo-lactic acid, acetic acid, CO_2, and ethyl alcohol.

L. mesenteroides belongs to the first group - the sucrose and pentose fermenters. It is the most common and the most active member of the Leuconostoc genus. It produces dextran from sucrose, and forms acid from glucose, fructose, galactose, mannose, xylose, arabinose, sucrose, generally forms acid from lactose, raffinose, salicin, mannitol, and rarely from dextrin, starch, inulin, sorbitol, rhamnose, or glycerol. The temperature range is from 5° C to 45° C, The optimum temperature being 21°C to 25°C .

Alford and McClesky (1942) found that 25°C was the optimum for slime production. At 37°C the organism did not produce slime.

The gum (slime, ropiness) or dextran formed by L. mesenteroides was studied by Levi, Hawkins, and Hibbert (1942). They showed that the dextran elaborated by the organism was made up of three glucosides:

(a) Tetramethyl glucoside

(b) Trimethyl glucoside

(c) Dimethyl glucoside

The ratio of tetra. to tri. to dimethyl glucoside was 1,3,1. Three of the linkages between the building units were 1-6 linkages. The remaining two were either 1-4 or 1-6 glucosidic linkages.

Duodoroff (1945) also demonstrated that the hexose

units of the dextran were joined through a 1-6 linkage, but the type of linkage was undetermined. Jeanes, Wilham, and Miers (1948) stated that the dextran was a polysaccharide having predominantly alpha 1-6 glucosidic linkages.

Isolation and Identification of *Leuconostoc mesenteroides*

Streaks were made on tomato juice agar and on yeast extract-glucose agar from the samples of ropy peaches obtained from the Michigan Agricultural Laboratory. The yeast extract-glucose agar gave the best growth so it was this medium which was used for the isolation of the organism. Yeast extract-glucose agar plates were streaked and single colonies were picked and streaked on another series of yeast extract-glucose agar plates. This was repeated six times. To further insure the purification of the organism single colonies were picked from the sixth plate and inoculated into 15 ml vials of yeast extract-glucose broth. The vials were well shaken and allowed to stand for twenty minutes. Streaks were then made on yeast extract-glucose agar plates. The dilution of a single colony through 15 ml of sterile yeast extract glucose broth and the resulting streaking gave greater assurance of the isolation of a pure strain.

When certain that a pure strain had been obtained slants of yeast extract-glucose agar were prepared and inoculated with eleven different colonies of the pure strain. The Manual of Methods for the Pure Culture Study of Bacteria recommends that a minimum of six isolates of the organism to be identified be run through the different tests for identification.

A gram stain of the organism was made from a twenty-four hour yeast extract-glucose broth culture. The stain showed gram positive cocci or very short rods in pairs or short chains.

Colonies twenty-four hours after streaking on yeast extract-glucose agar were uniformly round, pinpoint, translucent, raised, domeshaped, granular, and entire.

A can of peaches was obtained and macerated in a sterile Waring blendor. The macerated peaches and their juices were placed in small flasks and autoclaved. After autoclaving the flasks were inoculated with the isolates. Ropiness developed in all flasks in forty-eight hours. The ropy strings when stretched reached about one half inch or more before breaking. A gram stain revealed the same gram positive cocci in pairs or chains.

Morphological characteristics of the isolates:

(Size and shape) - Gram positive cocci

0.95 to 1.3 microns in diameter, occurring in pairs or in short chains.

Non-motile

Aerobic

Cultural characteristics:

Gelatin stab - Slight surface growth, no liquefaction.

Agar slant - Small, round, pinpoint, transparent, raised, dome-shaped.

Yeast extract-glucose agar (24 hours) - Round, pinpoint, translucent, raised, dome-shaped, granular, and entire.

Yeast extract-glucose broth - turbid.

Physiological characteristics

Litmus milk - no reduction

Indol test - negative

Nitrates not reduced to nitrites

Voges-Proskauer test - acetyl-methylcarbinol not formed.

Acid no gas in - xylose, arabinose, dextrin, maltose, galactose, lactose, raffinose, glucose, and sucrose.

Very slight acid and no gas in - mannose, glycerol, and starch.

No acid nor gas in - mannose, inulin, and mannitol.

Habitat:

Isolated from ropy canned peaches.

Checking the characteristics against Bergey's Manual of Determinative Bacteriology, the organism to which the above characteristics most nearly corresponded was L. mesenteroides. The only difference noted was that no acid was produced from mannose.

Summary

Leuconostoc mesenteroides was shown to be the causative organism of ropiness in samples of canned peaches.

TABLE I

CARBOHYDRATE UTILIZATION BY ORGANISMS ISOLATED FROM CANNED PEACHES

Carbohydrate	1	2	3	4	5	6	7	8	9	10	11	Gas Production
Xylose	a	a	a	a	a	a	a	a	a	a	a	-
Arabinose	a	a	a	a	a	a	a	a	a	a	a	-
Dextrin	a	a	a	a	a	a	a	a	a	a	a	-
Maltose	a	a	a	a	a	a	a	a	a	a	a	-
Galactose	a	a	a	a	a	a	a	a	a	a	a	-
Lactose	a	a	a	a	a	a	a	a	a	a	a	-
Rhamnose	-	s	-	-	s	s	s	-	-	s	-	-
Mannose	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	a	a	a	a	a	a	a	a	a	a	-
Dextrose	a	a	a	a	a	a	a	a	a	a	a	-
Inulin	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	a	-	-	-	-	-	-	s	-	-

Key: a = acid production, s = slight acid production
- = negative production.

TABLE II

ADDITIONAL PHYSIOLOGICAL TESTS ON THE ORGANISMS ISOLATED FROM CANNED PEACHES

Isolates	1	2	3	4	5	6	7	8	9	10	11
Indol	-	-	-	-	-	-	-	-	-	-	-
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-
Gelatin liquifaction	-	-	-	-	-	-	-	-	-	-	-
Litmus milk	-	-	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-	-	-

Literature cited

Alford, John A., and McClesky, C. S., (1942). Studies on Slime Production by Some Members of the Genus Leuconostoc. Jour. Bact. 43 ; 24-25.

Beck, E.C., and Chase, F.E., (1938). Some Experiments Dealing With Ropy Milk. Sci. Agric. (Ottawa) 19 ; 48-54.

Bergey's Manual of Determinative Bacteriology (1948) 6th Ed. Williams and Wilkins Company. Baltimore, Maryland.

Buchanan, R. E., and Hammer, B. W., (1915). Slimy and Ropy Milk. Iowa Agr. Exp. Sta. Res. Bull. 22 ; 211-295.

Cienkowski, L., (1874). Die Gallertbildungen des Zuckerubensaftes. Charkow. cited by Hucker and Pederson (1942) Food Res. 7 (6) ; 459-480.

Davis, J.G., (1935). Ropy Milk. Jour. Min. Agric. (Great Britain) 42 (7) ; 658-662.

Doudoroff, Micheal, (1945). On the Utilization and Synthesis of Sucrose and Related Compounds by Some Microorganisms. Fed. Proc. 4 (3) ; 241.

Edson, H. A., (1910). Buddy Sap. Vt. Agri. Expt. Sta. Bull. 151.

Edson, H.A., Jones, C. H., and Carpenter, C. W., (1912). Microorganisms of Maple Sap. Vt. Agri. Exp. Sta. Bull. 167.

Ehrenberg, (1840). Verhandl. d. Berl. Akad. 34 ; 202. cited by Kelly (1933) N. Y. (Geneva) Agr. Exp. Sta. Bull. 631.

Fabian, F.W., and Buskirk, H. H., (1935). Aerobacter aerogenes as a Cause of Ropiness in Maple Syrup. Ind. and Engr. Chem. 27 ; 349.

Fabian, F.W., and Nienhuis, A. L., (1934). Experimental Work on Cucumber Fermentation VI. Factors Influencing the Formation of Ropy Brine in Cucumber Fermentation. Tech. Bull. 140; Michigan Agr. Exp'l. Sta.

Feltz, Eugene, (1874). Del'accumulation de materes gelatineueses dans les presses continues. Sucrerie Indigene 9 ; 197-199. cited by Hucker and Pederson (1942). Food Res. 7 (6) ; 459-480.

Greene, R. A., Judd, E., and Marx, G.W., (1940). "Ropiness" in Tea Caused by Aerobacter aerogenes in a Water Supply. Amer. Jour. Pub. Health 30(6) ; 680.

Hammer, B. W., and Cordes, W. A., (1920). An Unusual Outbreak of Ropy Milk. Jour. of Dairy Sci. 3 ; 291.

Hohl, L.A., (1942). A Study of Organisms Found in Lactic Acid Fermentation of Lettuce. Food Res. 7 (4) ; 309-312.

Heideman, A. G., (1923). Abnormalities of Meat Curing Solutions. Viscid Brine: So-called "Ropy Pickle". Abst. Bact. 8 ; 14.

Horovitz-Vlasova, and Livshitz, M.I.,(1931). Experimental Study of the So-Called Sliminess of Salt Pork. Izvestiya Tzentral. Nauch. - Issledovatel. Inst. Pishehevoi Vkusovoi Prom., 42-46. Chem. Abs. 28 ; Jan-May 1931.

Horovitz-Vlasova, and Livshitz, M.I., (1931). Problem of Lipolytic Bacteria. Izveatiya Tzentral Nauch. - Issledovatel. Inst. Pishehevoi Vkusovoi Prom., 56-66. Chem. Abst. 28 ; Jan-May 1931.

Hucker, G. J., and Pederson, C.S.,(1930). Studies on the Coccaceae XVI. The Genus Leuconostoc. N.W. State (Geneva) Agri. Sta. Tech. Bull. 167 ; 1-80.

Jeanes, A., Wilham, C.A., and Miers, J. Cl, (1948). Preparation and Characterization of Dextran from Leuconostoc mesenteroides. Jour. Biochem. 176 ; 603-615.

Jubert, Paul, (1874). Note sur les gommes de sucreries. Sucrerie Indigene 9 ; 175-177, 199-200. cited by Anna Arrhenius, (1946) Inter. Sugar Jour. 48(574) ; 269-271.

Kayser, (1911). Compt. Rend. Acad. Sci. 152 ; 1422-1424. cited by Tanner, Microbiology of Foods p. 645. 2nd Ed. 1946. Garrard Press. Champaign, Ill.

Kircher, Juli, (1839). Ueber Mannit und Schleim aus runkelruben. Justis Liebigs Ann. Chem. 31 ; 337-339. cited by Hucker and Pederson, Food Res. 7 (6) ; 459-480. 1942.

Kratschmer and Niemilowicz, (1889). Vierteljahraschrift Nahr. u. Genussmtl. 4 ; 305. cited by Tanner, Microbioligy of Foods p. 707, 2nd Ed. Garrard Press. Champaign, Ill. 1946.

Laurent, (1885) Brussels Acad. Roy. de Belg. Bull. Ser. 3(10) ; 756. cited by Tanner, Microbiology of Foods, p. 707, 2nd Ed. Garrard Press. Champaign, Ill. 1946.

Levi, I., Hawkins, L., and Hibbert, H., (1942). Studies on Reactions Relating to Carbohydrates and Polysaccharides LXVI. Structure of the Dextran Synthesized by the Action of Leuconostoc mesenteroides on Sucrose. Amer. Chem. Soc. Jour. 64(8) ; 1950.

Liesenberg, C., and Zopf, W., (1892). Ueber den sogenannten Froshlaichpliz (Leuconostoc) er europaishen Rubenzucker-und der javanischen Rohrzuckerfabriken. Beitr. Physiol. u. Morphol. Nied. Organis. Liepzig Heft 1;1-29, Heft 2 ; 1-2. cited by Hucker and Pederson, Food Res. 7(6) ; 459-480, 1942.

Manual of Methods for the Pure Culture Study of Bacteria. Soc. of Amer. Bacteriologists. 1948-1949 Ed.,

Biotech. Publications. Geneva, New York.

M'Kenzie, D.A., Morgan, G.F.V., and Parker, E.M., (1943). A Case of Ropiness Due to Hay Contamination. Proc. Soc. Agri. Bact. (Great Britain) 1943, 34-35.

Pederson, C. S., (1930). Floral Changes in the Fermentation of Sauerkraut. N.Y. State (Geneva) Agri. Exp. Sta. Tech. Bull. 168 ; 3-38.

Pederson, C. S., (1931). Sauerkraut. N.Y. Agri. Exp. Sta. Bull. 595.

Prouty, C. C., (1943). A Ropy Milk Outbreak Caused by a Thermoduric Micrococcus. Jour. Milk Tech. 6(5) ; 263.

Rodenkirchen, J., (1939). Untersuchungen uber fadenziehenden Rahm. Zentralbl. Bakt. I, 144 ; 187-188.

Russell, H. L., (1898). "Sticky" or "Slimy" Bread and Its Cause. Wis. Agri. Exp. Sta. 15th Ann. Rept. 110.

Sarles, W. B., and Hammer, B.W., (1935). Species of Escherichia-Aerogenes Organisms Responsible for Some Defects in Dairy Products. Jour. of Bact. 25 ; 461-467.

Schroder, (1930). Deut. Tierarztl. Wchnschr. 38 ; 385-388. cited by Tanner, Microbiology of Foods, p. 858, 2nd Ed. Garrard Press. Champaign, Ill. 1946.

Spiegelberg, C. H., (1940). Some Factors in the Spoilage of Acid Canned Fruit. Food Res. 5(5) ; 439-455.

Stark, C. N., and Foter, M.J., (1931). The Characteristics of Organisms Causing Ropy Milk and the Importance of Feeds as their Source. Cornell Veterinarian 21 ; 109-113.

Tarnutzer, C. A., (1939). Ropy Chocolate Milk. Milk Dealer 28(10) ; 42, 84.

Van Tieghem, P., (1878). Sur la gomme de sucrerie. Ann. Sci. Nat. Bot. 6 ser. 7 : 180-203. cited by Hucker and Pederson, Food Res. 7(6) ; 459-480. 1942.

Vaughan, R. H., Douglas, H. C., and Gilliland, J. R., (1943). Production of Spanish Type Green Olives. Calif. Agr. Exp. Sta. Bull. 678 ; 1-82.