Studies on *Bacillus coagulans*

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CANNED evaporated milk is usually considered, for all practical purposes, to be sterile. If bacteria, or their spores, survive the sterilization process, they seldom grow in the canned product. Cases are on record, however, in which heat-resistant bacteria survived the heat processing and caused spoilage of the canned milk. Dr. Hammer was one of the first dairy bacteriologists in this country to investigate the spoilage of evaporated milk by heat-resistant bacteria.

In 1915 an outbreak of coagulation in evaporated milk occurred in an Iowa condensery. The bacteriological studies on the cause of this outbreak were reported by Hammer (3); and the causative organism, *Bacillus coagulans,* was thoroughly described. Cordes (1) found *B. coagulans* responsible for an outbreak of "flat-sours" in the evaporated milk packed by a condensery in a neighboring state. Sarles and Hammer (4) found *B. coagulans* to be the cause of a serious outbreak of coagulation in the evaporated milk packed by a Wisconsin condensery.

In each of these outbreaks the cans of milk which coagulated during storage had received, according to the plant records, very thorough heat treatment. It was reasonably certain that some of the milk which spoiled during the 1915 outbreak had been heated to 113.3° C. (236° F.) for 36 minutes. The cans of spoiled milk from which *B. coagulans* was isolated by Cordes in 1928, had been subjected to 114.4° C. (238° F.) for 20 minutes in a batch sterilizer. In the last outbreak studied, which occurred during the late summer and early fall months of 1930 and was reported in 1932, cans of milk which had been run through hot water at 97.8° C. (208° F.) for 24 minutes and then through a continuous cooker for 15 minutes at 117.8° C. (244° F.), showed, after several days' storage, coagulation caused by *B. coagulans*. These figures are given to illustrate that the heat resistance of this organism was high in each of the outbreaks studied.

The spoiled milk in each case usually showed a smooth, fairly firm curd with little whey. The cans of spoiled milk showed no signs of bulging, and no gas-escape was noted when they were opened. The quantity of gas present in cans of milk spoiled by *B. coagulans* was found to be similar to that in normal cans of milk. This gas was made up of about 15 percent carbon dioxide and 85 percent nitrogen. In cans of normal evaporated milk the gas was found to consist of 0.1 percent carbon dioxide and 99.9 percent nitrogen. The odor and taste of the spoiled milk was rather sour and cheesy but not at all disagreeable.

Isolation of the causative organism from cans of coagulated milk was usually not difficult because, in most cases, about 30,000 of *B. coagulans* per ml. were found by the plate count. This number, however, varied in different cans of spoiled milk from a few cells per ml. to over 9,000,000 per ml.

The original description of *B. coagulans,* made by Hammer in 1915 and corroborated and enlarged in 1932, is given in full below.

DESCRIPTION OF *BACILLUS COAGULANS*

MORPHOLOGY

Form and Size. Rods; 0.5 to 0.7 by 1.6 to 7.1 microns when grown on beef-infusion agar (24 hours at 37° C.); somewhat smaller when grown in milk (48 hours at 37° C.).

Arrangement. Singly and in short chains.

Motility. Motile; flagellation peritrichous.

Staining Reactions. Gram-positive in young cultures, often with distinct granulation; commonly gram-negative in old cultures although a few gram-positive cells sometimes persisted.

Spore Formation. In old beef-infusion agar slant cultures and in coagulated evaporated milk some cells contained spores. Preparations made from agar, or milk cultures grown under various incubation conditions, regularly showed spores in less than half of the cells. The spores were small, round, did not bulge the cells and were sub-terminal.

CULTURAL CHARACTERISTICS

Agar Slant. Beef-infusion and whey agars showed abundant, echinulate, white, nonviscid, shiny growth after two to three days at 37° C. Growth less abundant on standard agar.

Agar Stab. Beef-infusion agar and whey agars showed heavy, white, nonviscid, surface growth with some growth along the line of inoculation after two to three days at 37° C.

Agar Colony. After two to three days at 37° C. surface colonies on beef-infusion agar were shiny, white, nonviscid, round, about 1 to 2 mm. in diameter, with entire edge. Sub-surface colonies were round to oval, white, nonviscid, and smaller than the surface colonies.

Gelatin Stab. On whey gelatin at 37° C. growth occurred; gelatin not liquefied.

Broth. Turbidity with sediment.

Potato. Dirty white, shiny, nonviscid, spreading growth.

Litmus Milk. Litmus milk was reduced. Reduction was followed by coagulation and appearance of red band at top of milk. Red band increased in depth, curd contracted, expressing small amount of whey. No apparent proteolysis. Coagulation in four to eight days at 37° C. and in two to four days at 50° C.

BIOCHEMICAL FEATURES

Indol. Not produced.

Nitrates. Not reduced.

Action on Carbohydrates and Alcohols. Dextrin, galactose, glucose, glycerol, lactose, levulose, maltose, raffinose, salicin, sucrose and soluble starch fermented with the production of acid but no gas; adonitol, arabinose, dulcitol, inositol, inulin, mannitol and sorbitol not fermented; starch hydrolyzed.

Oxygen Relationship. Organism facultative; grew well aerobically.

Growth Temperatures. Grew well between 37° and 55° C.; poorly, if at all, at 20° C.

The total and volatile acidities of evaporated milk coagulated by this organism were studied. It was found that the abnormal milk showed total acidities of from 0.85 to 1.06 percent, while normal evaporated milk varied from 0.42 to 0.49 percent acid calculated as lactic acid. The principal acid formed by *B. coagulans* was found to be *d*-lactic acid. The small quantity of volatile acid produced by this organism was shown to be largely acetic, with a trace of propionic acid.

Evaporated milk spoiled by *B. coagulans* showed no evidence of proteolysis, but it was found to contain about twice the soluble nitrogen and more than double the amino nitrogen found in the normal product.

Among the problems left unsolved by the published work on *B. coagulans* were the factors influencing spore-formation and the relation of spore-formation to the heat resistance of the organism. Working under the author's direction, Elliker (2) has reported studies on these problems.

The nine strains of the organism used in this work were those isolated in 1930 by Hammer and the author.

Three of the nine strains produced very few spores under any conditions, despite the use of most of the known methods for inducing sporulation. The other six strains seemed to sporulate readily. These cultures formed spores more readily when cultivated on agar media than when cultivated in milk or broth. The optimum temperature for spore-formation was found to be 47° C.

Attempts to correlate spore-formation with the heat resistance of the various strains illustrated that, in general, those which produced spores readily were more resistant than those which formed few spores. Few spores could be demonstrated with the staining methods used in cultures grown in milk, but these milk cultures exhibited the same heat resistance as those grown on agar. The readily sporulating strains probably formed spores in milk, but our methods for demonstrating them were inadequate.

In conclusion, it should be pointed out that the original source of *B. coagulans* is not known, and that the knowledge concerning its heat resistance is quite inadequate. However, outbreaks of spoilage of evaporated milk due to this organism are apparently few in number, and may be prevented by proper plant practice.

LITERATURE CITED

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