Thermophilic Organisms Involved in Food Spoilage: Aciduric Flat-Sour Sporeforming Aerobes

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(Received for publication May 28, 1980)

ABSTRACT
This is an abbreviated review of the aciduric bacterium, Bacillus coagulans, and its relation to flat sour spoilage of medium acid foods. Spore heat resistance, lack of health hazard potential, identification and cultural requirements as well as spoilage prevention and heat processing are selectively reviewed. Eliminated from discussion are a large number of publications dealing with physiology, genetics and thermal death kinetics of the organism and its spores.

THE ORGANISM
The aciduric thermophiles that cause flat-sour spoilage are a group of strains with somewhat variable characteristics in the species Bacillus coagulans. The species is identified step-wise by the following test results: catalase-positive, Voges-Proskauer positive, growth in anaerobic agar (without glucose or Eh indicator) growth at 50 C and no growth in 7% NaCl. Other positive reactions useful in identification are: acid in Voges-Proskauer broth (pH 4.2 - 4.8) and growth in 0.02% NaN3 medium (J2). The organism is generally regarded as "facultatively thermophilic," growing in artificial media at 20-55 C (all strains) or 60 C (16 of 22 strains). Rods vary from 0.6 - 1 μm in diameter to 2.5 - 5.0 μm in length and thus overlap with other Bacillus species of thermophilic nature. Cells of some strains resemble Bacillus circulans in that they are long and slender and their sporangia are swollen. Other strains resemble Bacillus subtilis with shorter and thicker cells; sporangia are not bulged. Organisms differing from typical B. coagulans by growth at 65 C, and by failure to grow at pH 5 or anaerobically, obscure the distinctions between B. coagulans and Bacillus stearothermophilus or B. circulans (J1).

Belly and Brock (2) obtained 17 strains of an acidophilic bacterium from mats of the alga, Cyanidium caldarium, in effluents from hot springs in Yellowstone National Park. The organisms resemble B. coagulans in growth temperature range. They are facultatively thermophilic rods that grow between 30 and 60 C, with spores located terminally to sub-terminally. A sharp difference is that strains of Belly and Brock will grow as low as pH 2.0 up to 6.0 with an optimum between 3-4, whereas the lower limit of pH 4 and an optimum pH between 5-7 is displayed by the 22 strains designated as B. coagulans by Gordon et al. (J2).

B. coagulans displays sufficient variation that some strains were given different species designations in the past (22). Most familiar is Berry's facultative thermophile Bacillus thermoacidurans described in 1933 (4) and isolated from spoiled tomato juice. Another strain, causing coagulation of evaporated milk, had been described earlier, in 1915, by Hammer (J3). Hence, the validly published, legitimate name Bacillus coagulans is the correct taxon (5,12).

Accepted synonyms (3) for B. coagulans are: Bacillus thermoacidificans. Renco 1942 (20); Lactobacillus cereale. Olson, 1944 (18) and Bacillus dextrolacticus. Andersen and Werkman, 1940 (1). SOURCES
Spores of B. coagulans can be isolated from soil (23) though they are relatively scarce (J1). Denny and Bohrer (6) observed a range of 65-1100 spores/gram of unwashed tomatoes; they noted the spore load was directly proportional to the amount of adherent soil. The organism has been isolated from milk, cream and condensed milk. Chip board separators used in packaging of empty cans were reported (23) as a source of B. coagulans spores; they have been isolated from empty cans and the product lines, belts and runways of tomato processing plants.

CULTURAL REQUIREMENTS
The culture medium of choice for this group is the acidified proteose-peptone agar described by Stern et al. (24). Commonly known as thermoacidurans agar, it supports luxuriant growth of B. coagulans and generally gives a more abundant sporulation than ordinary plating media. Dextrose tryptone agar is useful as a nonselective medium for recovery of heat-treated spores.

Growth occurs optimally between 35 and 45 C in nutrient media of pH 5-7. However, spores were found incapable of germination and outgrowth in artificial media below pH 5.0 (19). Berry (4) and Stern et al. (24), among others, noted that growth in tomato juice (pH = 4.2) of acclimatized cultures occurs at room temperature and 37 C but not at 55 C. Further, recovery
of heat-damaged spores is greater at 37 than at 55 C. When propagating \( B. \ coagulans \) from tomato juice, an inhibitory effect is noted from the juice. For example, 1 ml of juice and about 20 ml of counting medium reduces the count by 1/5 and 3 ml of juice by 1/3, according to Stern et al. (24). Thus no more than 1 ml of heated or spoiled juice should be used per plate of recovery medium. This limitation, as well as the pH and temperature requirements, is incorporated into Speck's procedure for enumeration of aciduric flat-sour sporeformers (23).

**SPORE HEAT RESISTANCE**

Yokoya and York (28) studied several conditions influencing the thermal death rate of \( B. \ coagulans \) strain 43P. They found that a sporulation medium containing peptone, yeast extract and glucose with supplemental phosphate and 50 ppm of \( \text{MnSO}_4 \) gave a higher apparent heat resistance in neutral phosphate buffer than the same sporulation medium from which \( \text{MnSO}_4 \) was omitted. The respective \( D_{96} \) values obtained were 69 min and 45 min. D values were also significantly affected by the suspending menstruum and recovery media but less so.

Berry (4) reported an \( F \) value (destruction time in minutes at 250 F), \( Z = 14.5 \), of 0.33 in tomato juice, pH 4.5, and 3.3 in neutral phosphate buffer for 10⁷ spores/ml. Assuming logarithmic death, the graphically derived \( D_{250} \) values (time in minutes to inactivate 90% of spores heated) are 0.048 and 0.48, respectively. Studies of seven thermoacidurans types in the National Canners Association (NCA), now National Food Processors Association (NFPA), laboratory showed none were more resistant than 30 min at 212 F or 0.4 min at 250 F with \( Z = 23 \) in tomato juice or soup. The thermal death profile in tomato sauce was \( F = 0.57 \) and \( Z = 20 \) (17). El-Bisi and Ordal (9) measured death rate variations due to differing sporulation conditions, using \( B. \ coagulans \) ATCC strain 8038. They derived \( D_{199} \) values ranging from 3.8 to 24 in M/40 neutral phosphate solution. An important finding (10) was that higher incubation temperatures of sporulating cultures markedly increased heat resistance of the spores produced, e.g., 45-C incubation gave spores with \( D_{199} F = ca. 19 \), vs. 30-C incubation which yielded spores with \( D_{199} F = ca. 6 \) (M/40 phosphate buffer, pH 7).

**SPOILAGE**

During the early years of manufacture of tomato juice, incidents of spoilage occurred, characterized by "no-swell" containers, development of a fruity or medicinal flavor and a lowering of product pH by 0.3-0.5 unit (4). In view of the relatively heat resistant bacterial spores that could be present in their juice, canners eventually turned to "flash" processing in a tubular heat exchanger, cooling to fill temperature and heating closed cans for a brief time to kill organisms which might enter during filling (25). By 1941, Stern et al. (24) were able to report that "flat-sour" spoilage of tomato juice was of rare occurrence. However, sporadic outbreaks in canned juice and tomatoes continued to plague the industry until scientifically based controls superseded empirical practices.

**PREVENTION OF SPOILAGE**

A usually sufficient heat process may be overcome by excessive spore loads. For tomatoes and tomato juice, a ready source of \( B. \ coagulans \) spores is the soil adhering to the fruit. Various procedures for soaking and waterspraying of tomatoes both in-field and in-plant were developed over several years of study by NCA research personnel in cooperation with tomato canners (6-8, 15,16). Some important findings were: (a) a rolling spray wash most effectively removed soil and simultaneously reduced the aciduric spore count, (b) soak tank water temperature had to be kept below 80 F to prevent growth of spoilage organisms, (c) weather conditions influenced considerably the numbers of spores on tomatoes; lower counts were generally encountered after dry rather than wet weather and (d) higher aciduric spore loads resulted from machine rather than from hand harvesting but thorough washing reduced the difference from a 10-fold to about a 2-fold magnitude. Ingredients for canned tomato products such as tomatoes, pulp, puree and milk, cream or dry milk can be examined for aciduric spores as another means of spoilage prevention (23).

Routine sanitation and clean-up of food contact line equipment such as pipes, tanks and valves should be monitored visually whenever possible and by culturing the wash water, rinse water or material taken from these sites. Other more accessible line surfaces may be swab or impression plated directly on thermoacidurans agar for 50-C incubation.

Segner's caveat relative to leaker derived spoilage (21) bears repeating here. The chief cause of leaker spoilage is rough handling of filled containers that are still wet. Hence every effort should be made to reduce or prevent abuse. This means proper design, correct installation and appropriate maintenance of can handling equipment.

The acid stock foods such as catsup, pickles, maraschino cherries, olives, capers, etc. of pH less than 4 are not spoiled by \( B. \ coagulans \). Fruit juices, sauces and purees of \( pH < 4 \) also escape such spoilage though viable spores of \( B. \ coagulans \) may be present in the commercially sterile products. It is generally known that bacterial spores in products below \( pH 4.0 \) are not likely to germinate (27). Low acid canned foods (\( pH > 4.6 \)) are sometimes spoiled by \( B. \ coagulans \) due to leakage, post process. Heat processes applied are adequate to inactivate \( B. \ coagulans \) spores since they are designed to kill spores of \( C. \ sporogenes \), \( C. botulinum \) and \( C. \ nigrificans \), with wide safety margins of 5 to 12 times their \( D_{250} \) values. Underprocessing must be considered a possibility if a pack displays mesophilic flat-sour spoilage (26).
HEAT PROCESSING

In earlier years, canners tended toward a minimal process for tomato products and juice. However, such products could be heated considerably more without affecting general quality as judged by experienced technologists. For example, little quality difference was noted between juice heated 25 min at 212°F and that heated 20 min at 235°F (25). A process for tomato juice in current use calls for presterilization equivalent to 30 sec at 245°F, fill at 185°F, hold 2 min at 190°F using steam or hot water, (initial temperature of 160°F), and water-cool to about 100°F. The HTST presterilization step provides considerable lethality in the pH range of 4-4.5 with no detectable quality loss.

HEALTH HAZARD POTENTIAL

Metabolic end products of glucose fermentation by B. coagulans are mainly lactic acid plus small amounts of acetoin, ethanol, acetic acid and 2,3-butanediol (11). Nitrogenous end products have not been reported but toxicity studies indicate no health hazard. Hanson (14) fed eleven cultures of thermophilic bacteria grown in skim milk to experimental animals. His strains included B. coagulans Hammer. None of the species produced detectable toxic substances and the animals increased normally in weight both during and after the feeding experiment. Nevertheless, these results do not justify the presence of large numbers of thermophiles in foods since modern methods of heating and rapid cooling combined with appropriate ingredient specifications would allow proper control.

REFERENCES


JOURNAL OF FOOD PROTECTION. VOL. 44, FEBRUARY 1981