

Spoilage Bacteria in Canned Foods

I. Flat Sour Spoilage Bacteria in Canned Asparagus and the Thermal Death Time

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Bacillus stearothermophilus was found to be a cause of spoilage of asparagus canned in Taiwan. The F_{250} and Z values of the isolates were 14.2 min and 17.8 F (-7.9 C), respectively.

Flat sour spoilage occurs chiefly in low-acid foods such as peas, corn, and lima beans (6), but can also occur in medium-acid foods, e.g., spinach, green beans, asparagus, beets (1).

Following the mass production of canned asparagus in Taiwan in 1963, flat sour spoilage developed. Results are presented here on the biological characteristics and thermal resistance of strains of *Bacillus stearothermophilus* isolated from canned asparagus and related material.

MATERIALS AND METHODS

Isolation of the organisms. The following materials were examined for the presence of *B. stearothermophilus*: soil from the asparagus culture bed, raw asparagus, canned asparagus before it was sealed, and spoiled, canned asparagus which had a sour odor. We modified the methods of isolation of flat sour organisms described by Knock (8), and those recommended by the American Public Health Association (1). Dextrose-tryptone agar with bromocresol purple (DT medium; 1) in single and double strengths was used to isolate the flat sour organisms. Nutrient agar enriched with yeast extract and dextrose (YD medium) was used to maintain stock cultures (13).

The following procedures were used for isolating cultures. Soil extract (one part soil and four parts water) was filtered through gauze and mixed with an equal volume of molten, double-strength DT medium. While it was still hot, the mixture was autoclaved for 10 min at 110 C, and then poured into petri dishes. Raw asparagus was boiled 20 min in an equal volume of water, and the supernatant fluid was mixed with an equal volume of double-strength DT medium prior to being poured into petri dishes. Brine from canned asparagus was also mixed with double-strength medium, held at 100 C for 20 min, and poured into petri dishes. Brine with a sour odor, from canned asparagus incubated for 7 days at 55 C before being opened was boiled 20 min, and a 2-ml portion was mixed with 10 ml of single-strength DT medium. After it gelled, the agar was stratified with the same medium.

All plates were incubated for 72 hr at 55 C. Colonies with yellow halos were isolated for study.

Inoculation of asparagus. Spores of each isolate were prepared by the inoculation of 100 ml of DT broth (1) with 10 ml of a 24-hr culture. After incubation for 3 days at 55 C, the culture was heated at 100 C for 20 min to destroy vegetative cells. Asparagus in cans (net weight, 425 g; drained weight, 280 g; sugar, 0.3%; salt, 2.0%; pH 5.4) was inoculated with 10^8 spores per can, sealed, and then heat-processed. Duplicate cans were processed at 120 C for 6, 11, and 16 min, or at 116 C for 12, 18, and 24 min.

Identification of the isolated organisms. The morphology and physiology of the isolated strains were examined by the methods described in the *Manual of Microbiological Methods* (13) and *Fundamental Principles of Bacteriology* (12).

Determination of the thermal death time. Spores were produced as described previously, suspended in DT broth (pH 6.8), and then filtered through sterile cotton. Next, 2-ml quantities were introduced into thermal-death-time tubes (inner diameter, 7 mm; outer diameter, 9 mm; length, 150 mm) which were then sealed in a flame (3). One of the isolates, strain FRI 131, was used for all tests. For each variable, 10 replicate tubes were prepared, heated in a thermostatically controlled oil bath, and cooled in water. After being left for 4 days at 55 C, the tubes were observed for growth.

RESULTS

Six strains were isolated from canned asparagus (strains FRI 211 and 212) and related materials, e.g., brine from cans (FRI 131 and 132), raw asparagus (FRI 133), and soil from the asparagus culture bed (FRI 660). All strains caused flat sour spoilage in inoculated packs which were underprocessed (6 min at 120 C, or 12 min at 116 C) and were subsequently incubated at 55 C for 6 days. The spoiled cans had a turbid brine, a sour odor, and a pH of 4.4 to 4.6. Unspoiled controls, after the same incubation period, had a clear brine, a normal odor, and a pH of 5.0.

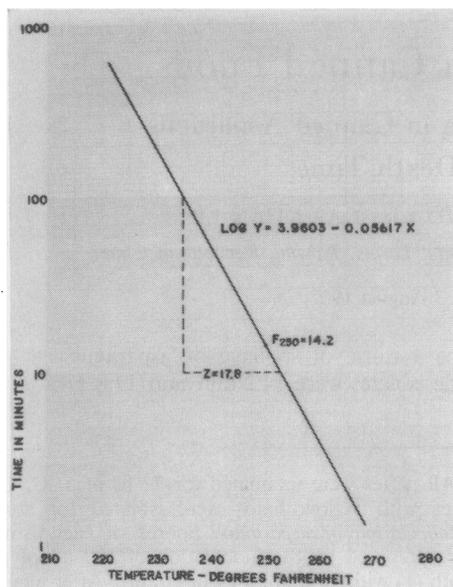


FIG. 1. Thermal-death-time curve.

The isolates were rod-shaped, 0.5×1.5 to 5.0μ in size, and were moderately motile. They formed terminal to subterminal spores 1.0×1.5 to 2.0μ in size. A few of the cells formed filaments; cells were gram-negative.

All strains hydrolyzed starch and liquefied gelatin but produced neither indole nor acetyl-methylcarbinol. Only one strain, FRI 212, failed to reduce nitrate to nitrite. All produced acid without gas from glucose, galactose, mannose, fructose, sucrose, maltose, starch, and salicin. Acid was not produced from xylose, arabinose, rhamnose, lactose, inulin, cellulose, mannitol, or glycerol. The optimal temperature for growth was between 55 and 65 C; the maximum was 72 C. No strain grew at 37 C.

The thermal-death-time curve of the strain FRI 131 (spore concentration, 1.4×10^3 per ml in DT broth, pH 6.8) is plotted in Fig. 1. The data showed that the F_{250} value was 14.2 min, and the Z value was 17.8 F (-7.9 C).

DISCUSSION

The flat sour spoilage is caused mainly by thermophilic spore-forming bacteria such as *B. thermoacidurans*, *B. coagulans*, *B. stearothermophilus*, and *B. pepo* (6, 7). Our experimental results, however, showed our strains to be *B. stearothermophilus*, as described in *Bergey's Manual of Determinative Bacteriology*.

These isolated strains have some different fermentation characteristics (Table 1) compared with the strains studied by Cameron and Esty (4).

TABLE 1. Comparison of our strains and the strains studied by Cameron and Esty

Medium acid produced in	Strains used ^a	Strains of Cameron and Esty			
		1792	1592	1476	1356
Arabinose.....	-	+	+	+	-
Fructose.....	+	+	+	-	+
Lactose.....	-	-	-	+	-
Salicin.....	+	-	-	+	-
Glycerol.....	-	+	+	-	+

^a All of our isolated strains.

Much work has been done on the thermal resistances of certain important food-spoilage bacteria and their spores. Different *F* values were found in the literature for *B. stearothermophilus*. Bigelow (2) found that the *F* value of *B. stearothermophilus* strain NCA 1518 was 5.2 min at 250 F (121 C). Reed, Bohrer, and Cameron (10), however, published an *F* value for this organism of 25.3 min in phosphate buffer (pH 7.0), and 44 min in pea puree. Fields and Finley (5) found the *F* value at 250 F (121 C) to be 7.6 min for a suspension of NCA 1518. The thermal death time (*F* value) for our isolated strain FRI 131 was 14.2 min at 250 F (121 C). The Z value was 17.8 F (-7.9 C). Some of these differences may have been due to traditionally recognized factors such as pH of medium, salt concentration, concentration of sugar and other carbohydrates, fat concentration, water content, and the age and concentration of spores as well as the method used to destroy spores (9, 11). Recently, Rotman and Fields (11) showed that the D_{250F} values for the smooth and rough variants spores were, respectively, 2.32 and 1.42 min even in the same strain of *B. stearothermophilus*.

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ERRATUM

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Volume 16, no. 1, page 45: change the second sentence of the abstract from

"The F_{250} and Z values of the isolates were 14.2 min and 17.8 F (-7.9 C), respectively."

to read

"The F_{250} and z values of the isolates were 14.2 min and 17.8 F, respectively."

page 46, column 1: change the last line above the DISCUSSION from

"the Z value was 17.8 F (-7.9 C)."

to read

"the z value was 17.8 F."

page 46, column 2, lines 14 and 15: change

"The Z value was 17.8 F (-7.9 C)."

to read

"The z value was 17.8 F."