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## FOOD HYGIENE STUDIES ON SPORE-FORMING GRAM-POSITIVE BACILLI

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(Summary of Master's thesis directed by Dr. S. HAMADA)

In as much as it has been recently reported that the outbreaks of food poisoning had been attributed to not only *Clostridium botulinum* but also to *Clos. perfringens*, *Bacillus cereus*, *Bac. firmus*, the significance of spore-forming Gram-positive bacilli in food materials have begun to attract the attention particularly of the persons concerned. But so far as the writer is aware, in most of the food hygiene reports on *Bacillus* or *Clostridium*, the food materials inspected are qualified as deteriorated materials or as presumed causal materials in outbreaks of food poisoning, and reports of fundamental studies on general normal food materials are few. Therefore, as one part of fundamental studies on normal food materials, the writer undertook to ascertain the frequency of presence of *Bacillus* and *Clostridium* in meat products, market milk and powdered skim-milk, and in swelled canned foods as a control; he also undertook studies on pathogenicity to mice, resistance to heat, and sensitivity to sorbic acid and nitrofurylacrylamide of the spore-forming Gram-positive bacilli isolated.

The results obtained are summarized as follows:

1. Spore-forming Gram-positive bacilli were detected at the rate of 84.6% (33/39) in meat products, 80.0% (48/60) in market milk, 100% (57/57) in powdered skim-milk and 29.9% (38/127) in swelled canned foods.

2. The number of *Bacillus* was greater than that of *Clostridium* in any food material inspected. Most strains of *Bacillus* found in meat products were *Bac. subtilis*, in market milk and powdered skim-milk *Bac. cereus*, and in swelled canned foods facultative anaerobic species. Most strains of *Clostridium* isolated from meat products were non-proteolytic but from swelled canned foods were proteolytic. Neither *Clos. botulinum* nor *Clos. perfringens* was detected.

3. Most strains of *Bac. cereus* and a few strains of the other species were intra-peritoneally pathogenic to mice, the pathogenicity of *Bac. cereus* being more powerful than that of the other species. The pathogenicity of these bacilli in broth culture was seemed mostly to be confined to one- to three-day cultures, but did not show any relation with sporulation.

No strains of *Clostridium* isolated were pathogenic to mice intra-peritoneally.

4. In the tests of resistance of *Bacillus* to heat, the fresh spores in phosphate buffer solution (number of spores:  $5 \times 10^6$  per ml) of most strains were resistant for 10 min. at 90°C or 100°C, but not for 30 min. at 100°C. The fresh spores (culture for 10 days at 37°C) of some strains could resist for 180 min. at 100°C or 10 min. at 110°C, but the old spores (maintained for 90 days at room temperature after being cultured for 10 days at 37°C) of some strains could not resist for 30 min. at 60°C or 20 min. at 80°C.

In the heat-resistance tests of fresh spores of *Clostridium*, 28 out of 55 strains could not resist for 10 min. at 100°C, while 23 strains could resist for 30 min. or more at 100°C. Most of the strains isolated from swelled canned foods were both proteolytic and strongly resistant to heat.

5. Each concentration allowed for use in foods (0.20% of sorbic acid and 0.0020% of nitrofurylacrylamide) could not effectively inhibit the growth of many species of *Bacillus*.

6. In bacilli investigated no correlation was observed between pathogenicity to mice, resistance to heat and sensitivity to preservative or bactericide.