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HOKKAIDO UNIVERSITY
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. There­
fore, 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.

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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.