Distribution of *Bacillus* Species in Hot Cooked Foods Kept in Heating Cabinets for Consumers

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Fifty samples of daily dishes (26 samples of croquettes, 10 of fried fish, 5 of hamburger steaks, 5 of meat balls and 4 of minced meat cutlets) kept hot in heating cabinets were used for a bacterial survey. Coliforms were detected in two of these foods (isolation rate 4.0%). Forty-eight samples of these foods (96%) were contaminated by *Bacillus* species, especially *Bacillus licheniformis*, *B. subtilis*, and *B. coagulans*.

Key words: hot cooked foods; heating cabinets; flat sour spoilage; total bacterial counts; coliforms; *Staphylococcus aureus*; thermophiles; *Bacillus* species

Some cooked daily dishes are being sold hot in open heating cabinets at supermarkets or retail stores in Japan, and are becoming increasingly used in daily life because of their convenience. Vending machines have also become popular, especially in the soft drinks industry. Nakayama and Shinya^{1), 2)} and York *et al.*³⁾ described the growth of thermophilic spoilage microorganisms, which produce either gaseous or flat sour spoilage in canned goods kept hot. These microorganisms thrive at the serving temperature (*ca.* 60°C) in vending machines. However, bacterial contamination of daily dishes kept hot in open heating cabinets remains obscure.

This study was done to determine the bacterial contamination of foods that are cooked, maintained in open heating cabinets, and sold throughout the day.

Materials and Methods

Samples of foods

Fifty samples of hot dishes that were obtained from October 1985 to January 1986 at several supermarkets, were examined for bacterial assay. The samples comprised 26 croquettes, 10 fried fish, 5 hamburger steaks, 5 meat balls, and 4 minced meat cutlets. They were bought just after the opening of the stores in the morning. These foods, each weighing 200~300 g, were packaged in styrofoam boats covered with plas-

tic and were kept hot in open heating cabinets that were equipped with thermometers (*ca.* 40~65°C). After their purchase, they were brought back to our laboratory in a shopping bag at the normal temperature, and they were analyzed within one hour.

Bacterial survey

A 10 g sample of each dish was homogenized in a sterile polyethylene bag with 90 ml of physiological saline solution. The homogenates were used to determine total bacterial counts, coliforms and *Staphylococcus aureus*. Total bacterial counts were done using the standard method agar for plate counts (Nissui Ltd., Tokyo, Japan) for mesophiles and thermophiles, which were incubated for 48 hr at 37°C and 55°C, respectively. Coliforms and *S. aureus* were determined by using the authorized method of the Food Sanitation Law in Japan, and these organisms were isolated on *Salmonella Shigella* Brom cresol purple (SSB) agar for *Enterobacteriaceae* (Nissui) and on mannitol salt agar containing egg yolk (Nissui) for *S. aureus*. The rest of the samples were kept hot at 55°C for 4hr and then were reassayed for thermophiles.

Isolation of Bacillus species

About ten colonies per sample from standard method agar plates were isolated randomly. These cultures were purified, grown on nutrient agar slants (Nissui) at 37°C and 55°C for 24hr,

Table 1. Total Bacterial Counts for Mesophiles and Detection of Coliforms and *Staphylococcus aureus* in Foods*¹

Food	No. of samples	Total bacterial counts (cells/g)				Coliforms* ²		<i>S. aureus</i> * ²	
		< 10	10 ¹⁻²	10 ²⁻³	10 ³⁻⁴	+	-	+	-
Croquette	26	1	6	12	7	2	24	2	24
Fried fish	10	0	2	4	4	0	10	1	9
Hamburger steak	5	0	2	3	0	0	5	0	5
Meat ball	5	0	2	2	1	0	5	0	5
Minced meat cutlet	4	0	0	4	0	0	4	1	3
Total	50	1	12	25	12	2	48	4* ³	46

*¹ The plates were incubated at 37°C.*² +: positive samples; -: negative samples*³ Negative in direct plating, but positive after enrichment with heart infusion broth containing 7.5% salt.**Table 2.** Total Bacterial Counts for Thermophiles*¹

Food	No. of samples	Total bacterial counts (cells/g)* ²					
		< 10	10 ¹⁻²	10 ²⁻³	10 ³⁻⁴	10 ⁴⁻⁵	10 ⁵ <
Croquette	26	4	16	6 (1)	0 (11)	0 (14)	0 (0)
Fried fish	10	6	2	2 (2)	0 (5)	0 (1)	0 (2)
Hamburger steak	5	1	3	1 (1)	0 (1)	0 (2)	0 (1)
Meat ball	5	3	2	0 (3)	0 (2)	0 (0)	0 (0)
Minced meat cutlet	4	0	3	1 (2)	0 (1)	0 (1)	0 (0)
Total	50	14	26	10 (9)	0 (20)	0 (18)	0 (3)

*¹ The plates were incubated at 55°C.*² Number of samples kept hot at 55°C for 4 hr is shown in parentheses.

then classified as either mesophile or thermophile. The isolates were further identified as *Bacillus* species using the following tests: Gram-stain, shape, formation of spores, catalase reaction, and other conventional tests⁴⁾⁻⁶⁾.

Results and Discussion

Bacterial survey

Table 1 shows total bacterial counts, coliforms, and *S. aureus* in food samples. According to food hygienic criteria of lunch and daily dishes in Japan, total bacterial counts are to be less than 10⁵ cells per gram of food, and coliforms are not to be detected. All samples met the criteria for total bacterial count; however, two of the fifty foods contained coliforms (isolation rate 4.0%). The number of coliforms was 5 × 10 and 1.2 × 10² per gram, respectively. One food contained *Klebsiella pneumoniae* and the other had *Escherichia coli* and *Enterobacter cloacae*. *S. aureus* was not detected by direct plating, but was found in four samples when the

medium was enriched with heart infusion broth (Nissui) containing 7.5% salt. This organism produced the enterotoxins A and B. This fact has resulted in special interest from food authorities, who stress the importance of intensive control of food hygiene in these foods. The results suggest that special hygienic risks, such as infections with pathogenic bacteria, need to be considered in connection with the sale of such hot cooked foods.

Thermophiles are defined as bacteria which grow at 55°C or over^{7), 8)}. Judging from the thermophilic bacterial counts of foods kept hot at 55°C for 4 hr (Table 2), the results show that these organisms increase in number the longer the foods sit in the heating cabinets. Therefore indications not only of the date of preparation, but also of the time the food was prepared are necessary for consumers.

Isolation of *Bacillus* species

Five hundred and thirty-two strains were isolated from foods, and 353 of them (66.4%)

were identified as *Bacillus*. The others were gram-positive cocci, gram-negative non-sporing rods and yeasts which thrive at 37°C, but not at 55°C. Table 3 shows the *Bacillus* species of the 353 strains isolated from foods. Different *Bacillus* species were identified and Table 4 shows their distribution in foods. The predominant

Table 3. *Bacillus* Species of the 353 Strains Isolated from Foods

Species	No. of strains (%)
<i>B. licheniformis</i> ^a	178 (50.4)
<i>B. subtilis</i> ^b	59 (16.7)
<i>B. megaterium</i> ^b	22 (6.2)
<i>B. sphaericus</i> ^b	18 (5.1)
<i>B. coagulans</i> ^a	17 (4.8)
<i>B. brevis</i> ^a	12 (3.4)
<i>B. firmus</i> ^b	10 (2.8)
<i>B. pumilus</i> ^b	8 (2.3)
<i>B. acidocaldarius</i> ^c	7 (2.0)
<i>B. cereus</i> ^b	7 (2.0)
<i>B. stearothermophilus</i> ^c	6 (1.7)
<i>B. circulans</i> ^b	4 (1.1)
<i>B. polymyxa</i> ^b	3 (0.8)
<i>B. laterosporus</i> ^b	2 (0.6)

^a Facultative thermophiles grow at both 37°C and 55°C.

^b Mesophiles grow at 37°C, but not at 55°C.

^c Strict thermophiles grow at 55°C, but not at 37°C.

species from foods were *B. licheniformis*, *B. subtilis* and *B. coagulans*. Similar results were reported in milk powder⁵⁾. In general thermophiles are divided into facultative thermophiles which grow at both 37°C and 55°C, and strict thermophiles which grow at 55°C, but not at 37°C⁸⁾. The facultative thermophiles isolated from foods were *B. licheniformis*, *B. coagulans* and *B. brevis*, the strict thermophiles were *B. acidocaldarius* and *B. stearothermophilus*, and the others were mesophilic *Bacillus*. The pathogenicities of these organisms are not yet clear (only some strains of *B. cereus* are classified as pathogens), but 10⁸ *B. stearothermophilus* per g were detected in a case of food-borne illness that resulted from boiled rice that was kept hot at about 70°C in a rice cooker in 1979⁹⁾. The presence of these bacilli may be due to the survival of heat-resistant spores in the raw materials. These may eventually result in spoilage of foods kept hot in heating cabinets; however, no cases of spoilage have yet been reported. Thermophilic bacteria, such as *B. coagulans* and *B. stearothermophilus* often can produce thermostable enzymes^{10), 11)} and cause flat sour spoilage of foods^{1)~3)}.

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Table 4. Distribution of *Bacillus* Species in Foods

Species	No. of positive isolations (%) in:				
	Croquette (n=26)	Fried fish (n=10)	Hamburger steak (n=5)	Meat ball (n=5)	Minced meat cutlet (n=4)
<i>B. licheniformis</i> ^a	20 (76.9)	10 (100)	4 (80.0)	3 (60.0)	2 (50.0)
<i>B. subtilis</i> ^b	11 (42.3)	5 (50.0)	3 (60.0)	3 (60.0)	4 (100)
<i>B. coagulans</i> ^a	6 (23.1)	2 (20.0)	3 (60.0)	0	0
<i>B. megaterium</i> ^b	7 (26.9)	0	1 (20.0)	0	0
<i>B. sphaericus</i> ^b	4 (15.4)	0	3 (60.0)	0	0
<i>B. brevis</i> ^a	4 (15.4)	0	0	3 (60.0)	0
<i>B. firmus</i> ^b	5 (19.2)	0	0	0	2 (50.0)
<i>B. pumilus</i> ^b	2 (7.7)	3 (30.0)	0	0	1 (25.0)
<i>B. acidocaldarius</i> ^c	5 (19.2)	0	1 (20.0)	0	0
<i>B. cereus</i> ^b	4 (15.4)	0	0	0	0
<i>B. polymyxa</i> ^b	3 (11.5)	0	0	0	0
<i>B. circulans</i> ^b	2 (7.7)	0	0	1 (20.0)	0
<i>B. stearothermophilus</i> ^c	2 (7.7)	0	0	0	1 (25.0)
<i>B. laterosporus</i> ^b	1 (3.8)	0	0	0	0

^{a, b, c} See Table 3.

study.

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