

Incidence and Levels of *Bacillus cereus* in Processed Spices

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ABSTRACT

Spices purchased by the Army, Navy, Marines, and Air Force were tested for incidence and levels of *Bacillus cereus*. One hundred and ten processed spices, including bay leaves, red pepper, chili powder, cinnamon, garlic powder, mustard powder, and oregano were tested. *Bacillus cereus* was found in 53% of the spices and counts ranged from 50 to 8500 per gram. Eighty-nine percent (88/99) of the isolates tested were toxigenic in rabbits, by the vascular permeability assay, and toxigenic *B. cereus* was found in each kind of spice. These data have significant implications for food safety and sanitation and for fumigation of spices by gas, or irradiation.

During a recent investigation of the microbiology of processed spices (10) appearance of *Bacillus cereus* type colonies was noted on aerobic plate count plates and this observation prompted further investigation. In addition to determining the microbiological safety of spices purchased by the military, examination of spices for *B. cereus* has important implications for writing military specifications and for the possible application of irradiation technology for radacidation (reduction of specific pathogens), or radappertization (destruction of all organisms) of spices.

Bacillus cereus has been recognized as the etiological agent in food poisoning outbreaks in Europe as far back as 1906 (4). In Hungary, it was ranked as the third most common cause of food poisoning during the period 1960-1966 (4). In the following 2 years it was responsible for 15.2% of all cases of food poisoning of known etiology (4). It is interesting to note that the high incidence of *B. cereus* food poisoning in meat was attributed to the Hungarian custom of highly seasoning meat dishes with spices which often contain large numbers of aerobic sporeformers. Numerous reports of *B. cereus* foodborne illness in Europe have been cited by Goepfert et al. (4).

In the United Kingdom, 12 reports were made of *B. cereus* food poisoning in Chinese restaurants between 1971 and 1973. Fried or boiled rice were implicated in all of the outbreaks (13).

Only 7 outbreaks of *B. cereus* foodborne illness were reported in the United States between 1968 and 1973 (13). The most recent outbreak occurred in 1975 in a family of four which purchased a meal at a fast food restaurant. Mashed potatoes from the suspect meal contained 1.8×10^7 *B. cereus* per gram with no other bacterial pathogens isolated (14).

Although a selective medium (MYP) for identification of *B. cereus* was developed by Mossel et al. in 1967 (9) and another (KG Agar) by Kim and Goepfert in 1971 (7), there have been very few reports of *B. cereus* foodborne illness in the United States and very few foods have been surveyed to determine the incidence and levels of this organism in our food supply. One study by Kim and Goepfert in 1970 found *B. cereus* in 25.3% of 170 selected dried products (8).

This investigation was undertaken to determine the safety of spices procured by the military and to ascertain the incidence and levels of *B. cereus* in spices for the purpose of writing microbiological specifications.

MATERIALS AND METHODS

Number and source of samples

One hundred and ten samples of the same spices studied earlier (10) were examined. The spices were received from 16 military bases, including the Army, Navy, Marines, and Air Force, located in different geographical areas of the United States. Spices were purchased from local supermarkets by each base and represented 10 different processors. Spices were stored in their containers at 23 C for approximately 1 year.

Preparation of samples

Samples were prepared as reported earlier (10).

Inhibition of bacterial growth by spices

It was previously determined that the spices were not inhibitory to bacterial growth at the concentration tested (10).

Media

KG agar (an egg yolk-polymyxin medium) was prepared according to Kim and Goepfert (7). Each batch of medium was tested with *B. cereus* strain B6AC (University of Wisconsin; originally from D. A. A. Mossel, the Netherlands) to determine typical growth characteristics of the organism.

B. cereus count

One-tenth milliliter of dilutions ranging from 10^{-1} to 10^{-5} was spread on the surface of duplicate plates of KG agar. Plates were incubated at 32 C for 24 h. Only typical colonies (rough, flat, dry, round or irregularly shaped, ground glass appearing, translucent to creamy white with a pink-red background) surrounded by a zone of turbidity (7, 9) were counted. Five representative colonies were examined microscopically for large celled, Group I bacilli (5, 11, 12), centrally located spore within the sporangium, and absence of parasporal inclusion bodies. Motility was determined in cystine trypticase agar incubated at 32 C for 24 h.

Demonstration of enterotoxigenicity

Ninety-nine typical isolates of *B. cereus* were cultured in the following manner: 0.4 ml of a 24-h old trypticase soy broth culture was

transferred to 125-ml shake flasks containing 40 ml of HY case (S. F. Sheffield, 2%), yeast extract (2%), Na₂HPO₄ (0.3%), Dextrose (0.4%), and 1 ml per liter of trace minerals (5.0% MgSO₄, 0.5% MnSO₄ and 0.5% Fe citrate). The medium was adjusted to pH 8.0. Cultures were shaken at 200 rpm, in a 1/2-inch circular orbit, in a constant temperature water bath at 37 C and incubated for approximately 6 h. The pH was maintained above 7.0 at all times by adding 0.5 N NaOH. Cultures were then centrifuged at 7,900 × g for 20 min. The supernatant fluid was recovered and stored at 4 C overnight.

The enterotoxigenicity of each isolate was assessed by testing supernatant fluid from each culture for activity in the vascular permeability assay of Glatz et al. (3). Female New Zealand white rabbits, weighing 2.0-2.5 kg, were used. Two determinations on each of three rabbits were averaged for each test sample. A *B. cereus* isolate was considered an enterotoxin producer when the zone of activity averaged greater than 15 mm². Sterile medium, which was used as a negative control, produced a reaction area which averaged 1 mm², or less.

RESULTS

Bay leaves

Eleven samples of bay leaves were analyzed. *B. cereus* was found in seven samples and counts ranged from 50 to 275/g (Table 1). Three samples had counts between 100 and 275/g. Eighty-two percent (9/11) of the isolates tested were toxigenic.

Cayenne pepper

Eighteen samples of ground cayenne pepper were analyzed. *B. cereus* was found in 12 samples and counts ranged from 50 to 3500/g (Table 1). Eleven samples had counts greater than 100/g and four samples had counts greater than 1000/g. Ninety six percent (24/25) of the isolates tested were toxigenic.

Chili powder

Sixteen samples of chili powder were analyzed. *B. cereus* was found in only four samples and the counts ranged from 50 to 500/g (Table 1). Three samples had counts between 100 and 500/g. All isolates tested (4/4) were toxigenic.

Cinnamon

Sixteen samples of ground cinnamon were analyzed. *B. cereus* counts ranged from 50 to 8500/g and was found in all samples except 1 (Table 1). Twelve samples had counts greater than 100/g and six samples had counts greater than 1000/g. Eighty three percent (24/29) of the isolates tested were toxigenic.

Garlic powder

Seventeen samples of garlic powder were analyzed. *B. cereus* was found in only five samples and counts ranged from 50 to 1000/g (Table 1). Three samples had counts greater than 500/g. One hundred percent (11/11) of the isolates tested were toxigenic.

Mustard powder

Fourteen samples of mustard powder were analyzed. *B. cereus* was found in only one sample and only one colony was isolated from the 1:10 dilution. The resulting count was 50/g (Table 1). The single isolate was toxigenic.

Oregano

Eighteen samples of oregano were analyzed. *B. cereus* was found in 14 samples and counts ranged from 50 to 3800/g (Table 1). Twelve samples had counts greater than 100/g and five had counts greater than 1000/g. Eighty three percent (15/18) of the isolates tested were toxigenic.

DISCUSSION

Bacillus cereus was found in 53% (58/110) of the spices analyzed and in each kind of spice. The incidence was higher than previously found in spices (40%) and more than double the incidence found in selected dry products (8). Counts ranged from 50 to 8500/g, but most of the spices (59%) had counts less than 100/g (Table 1). Only 15 samples (13.6%) had counts greater than 1000/g. No counts were obtained from 52 samples (47%) at the lowest dilution (1:10) and were reported as less than 100/g, since 0.1 ml of the 1:10 dilution (0.01 g of spice) was spread on each plate. Eighty nine percent (88/99) of the isolates tested produced enterotoxin (Table 1).

KG agar is considered a presumptive medium for *B. cereus* because it is not completely selective for the organism (7). However, it is a differential medium and colonies of *B. cereus* are easily distinguished from other contaminants. The difficulty one has with the medium is discerning the "turbid zone" (egg yolk reaction) around a colony even on plates which are not crowded with other contaminating colonies. We found that the egg yolk reaction was most easily discerned by holding the plate up to the ceiling light. In a few instances when plates

TABLE 1. *B. cereus* in processed spices

Spices	No. of samples	Range of counts/g	Percent isolates toxigenic	Number of samples containing (per gram)						
				50	<100 ^a	100-500	501-1000	1001-5000	5001 to 10,000	>10,000
Bay leaves	11	50 to 275	82 (9/11) ^b	4	4	3	0	0	0	0
Cayenne (Red) pepper	18	50 to 3500	96 (24/25)	1	6	4	3	4	0	0
Chili powder	16	50 to 500	100 (4/4)	1	12	3	0	0	0	0
Cinnamon	16	50 to 8500	83 (24/29)	3	1	5	1	4	2	0
Garlic powder	17	50 to 1000	100 (11/11)	1	12	1	3	0	0	0
Mustard powder	14	<100	100 (1/1)	1	13	0	0	0	0	0
Oregano	18	50 to 3800	83 (15/18)	2	4	4	3	5	0	0
Total	110	50-8500	89 (88/99)	13	52	20	10	13	2	0

^aNo counts per gram at 1:100 dilution.

^bNumber positive over total number of isolates.

were overly crowded with other bacterial colonies, suspected *B. cereus* colonies had to be transferred to fresh KG agar plates for confirmation and isolation.

Only colonies which exhibited typical morphology (7, 9) and were also positive for the egg yolk turbidity factor were counted as *B. cereus*. It was observed that typical colonies were always positive for the egg yolk turbidity factor.

Because aberrant and deviant strains of *B. cereus* are common, no attempt was made to characterize isolates by the usual biochemical tests (nitrate reduction, hydrolysis of starch and gelatin, acetylmethylcarbinol production, and anaerobic utilization of glucose.) The usefulness of these tests is questionable because, in addition to being time consuming they were often found to be erratic (7). Consequently, in the interest of saving time and effort, confirmation was simplified by examining five colonies from each plate microscopically, for large celled, Group I bacilli (5, 11, 12). Motility in cystine trypticase agar was also observed. Absence of parasporal inclusion bodies within the sporangium excluded *Bacillus thuringiensis* and absence of rhizoidal growth on KG agar excluded *Bacillus mycoides*. Egg yolk reaction excluded *Bacillus megaterium*. All isolates were motile which excluded *Bacillus anthracis* and *B. mycoides*.

With the exclusion of these closely related organisms the demonstration of enterotoxigenicity in rabbits served as additional confirmation of *B. cereus*, since only *B. cereus*, *B. thuringiensis*, and *B. mycoides*, of the bacilli tested by Glatz et al., produced vascular permeability factor activity (2, 3). However, these studies (2, 3) as well as this report, also indicate that 10 to 12% of the *B. cereus* strains tested may not elicit toxigenic activity. Of the 11% isolates which were not toxigenic by our criteria, only three failed to give any response. The remaining isolates gave measurable zones of activity, but they were less than 6 mm² and were considered negative.

These findings and our earlier report (10) point out that spices may be a source of contamination in the kitchen, and may introduce significant numbers of bacilli into food. Under certain circumstances *B. cereus* could multiply sufficiently to cause food poisoning. For example, foods which are highly seasoned such as Hungarian meat dishes (4), and particularly foods seasoned after cooking, such as Pommes de terre duchesse (6), may, if held at room temperature for several

hours, harbor sufficient *B. cereus* to cause illness.

It is important, therefore, that food service personnel be aware of the source of *B. cereus* and other pathogens, so that they can avoid some of the major causes of food poisoning outbreaks; i.e., improper refrigeration of foods, inadequate cooking of foods, allowing foods to remain at warm (bacterial incubation) temperatures, incorporating raw (contaminated) ingredients into foods that receive no further cooking, and cross contamination of cooked foods with contaminated raw foods (1).

While a potential health problem may be associated with these spices because of the presence of *Clostridium perfringens* (10) as well as *B. cereus*, foods which are seasoned would have to be mishandled and abused before a health hazard could actually exist.

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