Journal of Food Protection, Vol. 50, No. 1, Pages 62-65 (January 1987)
Copyright© International Association of Milk, Food and Environmental Sanitarians

Bacillus cereus Contamination of Seeds and Vegetable Sprouts Grown in a Home Sprouting Kit

STANLEY M. HARMON*, DONALD A. KAUTTER, and HAIM M. SOLOMON

Division of Microbiology, Food and Drug Administration, Washington, D.C. 20204

(Received for publication June 27, 1986)

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/50/1/62/1656061/0362-028x-50_1_62.pdf by guest on 28 September 2022

ABSTRACT

Sprouting seeds (alfalfa, mung bean and wheat) were purchased at local health food stores and examined for *Bacillus cereus* by the official AOAC method. Of 98 units collected, 56 (57%) were positive for *B. cereus* at levels ranging from 3 to >500 per g. Population levels of *B. cereus* on sprouts grown from naturally contaminated seeds in a home sprouting kit ranged from a mean of $\log_{10}$ 3.72 for alfalfa to 5.39 for wheat; the $\log_{10}$ mean for mung bean sprouts was 4.52. Washing contaminated sprouts for 10 min with warm tap water as recommended by the manufacturer of the sprouting kits reduced the *B. cereus* count for mung bean sprouts by approximately one log unit but was less effective for wheat sprouts. *B. cereus* populations large enough to cause food poisoning ($>10^5$/g) frequently remained on wheat sprouts even after three wash cycles, and significant numbers of viable *B. cereus* remained on wheat sprouts even after cooking for 20 min.

Although only a few food poisoning outbreaks in the United States have been attributed to consumption of vegetable sprouts, the potential for raw sprouts to serve as vehicles for transmission of pathogenic microbes has been recognized for several years (*1,2,7;* Park et al., 1981, Abstr. Annu. Meet. Can. Soc. Microbiol.; Park et al. 1983, Abstr. Annu. Meet. Can. Inst. Food. Sci.). Andrews et al. (*1*) showed that populations of bacteria increased dramatically on mung bean and alfalfa sprouts during normal sprouting and that *Salmonella* quickly reached hazardous levels on sprouts grown from inoculated seeds. Park et al. (Abstr. Annu. Meet. Can. Inst. Food Sci., 1981) have reported similar findings with *Klebsiella pneumoniae* on alfalfa and bean sprouts. In 1973, a food poisoning outbreak in Houston, Texas was traced to consumption of vegetable sprouts contaminated with *Bacillus cereus (7).* The symptoms experienced by the victims suggest that the illness was caused by an emetic strain. An investigation of factors contributing to the outbreak showed that *B. cereus* proliferated from about 100 per g on seeds supplied with the incriminated home sprouting kit to several million per g during normal sprouting (*7*). Because it was thought that the design of the sprouting kit used to grow the implicated sprouts may have contributed to the outbreak, the kit was temporarily banned by the Food and Drug Administration; however, it continues to be sold without sprouting seeds. Raw sprouts continue to be popular for use in salads, however, and so we decided to ascertain the incidence of *B. cereus* contamination on some popular sprouting seeds and to determine the potential of this organism to proliferate during sprouting of different types of vegetable seeds.

A survey of health food stores in the Washington, DC area revealed that mung beans and alfalfa are probably the most widely available commercially, and mung beans, alfalfa and wheat are the most popular seeds sold for home sprouting. Therefore, we selected these three types of seeds for our experiments. Mustard seeds, also implicated in the Houston outbreak, were unavailable. A total of 98 units of 8 different brands was purchased from local health food stores and tested for *B. cereus* contamination using the official AOAC method (*4*). Naturally contaminated and artificially inoculated seeds were sprouted in the Biosta Miracle Sprouter kit, which, except for color (green vs. clear), was identical to the kit implicated in the Houston outbreak. The sprouting technique recommended by the distributor of the kits was followed, and the sprouts were examined quantitatively for *B. cereus*. The effect of washing and heating to simulate the effects of cooking on *B. cereus* populations on the sprouts was also determined.

MATERIALS AND METHODS

Sprouting seeds

Sprouting seeds were purchased either in bulk or in prepackaged units of 1.5 to 2.0 lb. at health food stores in the Washington, DC area. Several different brands of alfalfa, mung beans and wheat were included. A quantity of each type sufficient to complete a particular experiment was purchased at the same time. The lots differed considerably in general cleanliness but no attempt was made to clean the seeds before use other than to remove stones or other obviously foreign material. When necessary, the contents of two or more packages were combined and mixed thoroughly to provide starting material. Five packages each of two popular brands of mixed seeds, which consisted mostly of mung beans and alfalfa seeds labeled

"spicy sandwich" or "spicy salad" mixture, were also tested for *B. cereus* contamination.

Sprouting kit

The Biosta Miracle Sprouter (Miracle Exclusives Inc., Locust Valley, NY) appeared to have a somewhat greater potential for promoting bacterial growth than the common Mason jar type. The Miracle Sprouter is a four-tiered, plastic cylinder made up of three trays (bowls) for sprouting seeds and a bottom bowl for collecting excess water. We used only one sprouting tray per unit to prevent cross contamination between sprouting trays. Between experiments the kits were scrubbed thoroughly with detergent solution and a stiff hand brush, and then sanitized with 0.5% sodium hypochlorite/detergent. The effectiveness of the washing and sanitizing procedure was tested by rinsing two kits with sterile distilled water and culturing the rinse water in Trypticase-soy polymyxin broth to detect spore formers. Unless the rinse water was negative, the cleansing and sanitizing procedures were repeated.

Cultures

The *B. cereus* cultures used for inoculating seeds were isolated from foods implicated in food poisoning outbreaks. Strains F4810/72, F4433/73, F4552/75, F4165/75 and F2969/77 were obtained from Richard Gilbert, Food Hygiene Laboratory (London). Strains B4ac, 19668 and 30020 were supplied by J. M. Goepfert, Food Research Institute, University of Wisconsin, Madison. Strains MB-31 and MB-34 were isolated in our laboratory from mung bean sprouts. Stock cultrues were maintained on nutrient agar slants stored at 4°C. Spore stocks were produced by culturing on nutrient agar slants at 30°C. Cultures were checked for spores by phase contrast microscopy and the growth was removed aseptically in sterile distilled water and washed twice by centrifugation for 20 min at 10,000 × *g*. Viable spore counts were made by heating 1 ml of the spore suspension for 20 min at 75°C in a water bath and plating on mannitol-egg yolk-polymyxin (MYP) agar (*4*).

Sprouting procedure

The sprouting procedure was similar to that described by Andrews et al. (*1*). A 20-g portion of seeds (10 g of alfalfa) was spread evenly onto the corrugated bottom of each seed bowl and watered with 500 ml of tepid distilled water. Water was allowed to drain from the seed bowl and discarded. The seeds were kept at room temperature and watered twice daily until sprouts were mature. This required about 3 d for alfalfa and mung beans but only 2 d for wheat.

To determine the effect of specific contamination levels, *B. cereus* spores were added to the initial water in some experiments at levels of 100-200/g for wheat seeds and 500/g for alfalfa and mung beans. A 500-ml volume of plain distilled water was used for all subsequent waterings. After 2 or 3 d at room temperature, depending on the type of seeds, sprouts were collected and examined for *B. cereus*.

Washing and cooking procedures

To determine the effects of washing and cooking on *B. cereus* counts, a 25-g portion of each batch of sprouts was transferred to an 8-inch No. 8 sieve and washed three times for 5 min with a stong spray of cold tap water. The sprouts were redistributed on the sieve twice between washings. To determine the effect of cooking sprouts on *B. cereus* counts, washed and unwashed sprouts (25 g each) were transferred to separate beakers containing 100 ml of sterile distilled water and immersed in a water bath at 98°C. The contents of each beaker were stirred frequently until the internal temperature reached 95°C. After 20 min, the beakers were removed and cooled in cold tap water. The contents of each beaker were combined with 125 ml of Butterfield's buffer in a Waring blender jar and homogenized for analysis.

Bacteriological analysis

A 25-g portion of sprouts was homogenized in 225 ml of Butterfield's buffer and plated on MYP agar as specified in the official AOAC method (*4*). Isolates were identified as *B. cereus* by the method of Harmon (*3*). To detect contamination on dry seeds, 20-g portions were aseptically weighed into 125-ml Wheaton storage bottles containing 100 ml of Butterfield's buffer. The bottles were shaken vigorously by hand for 2 min, allowed to stand for 5 min and shaken again for 2 min. The rinse water was decanted aseptically and tested for *B. cereus* spores using the most probable number technique in Trypticase-soy polymyxin broth. Presumptive positive tubes were streaked on MYP agar plates and colonies were identified as *B. cereus* (*3*). To enhance recovery of spore formers, the inoculated tubes were held in a water bath at 55°C for 1 h before incubation.

RESULTS AND DISCUSSION

Natural contamination

Of the 98 units of unsprouted seeds examined, 56 (57%) were contaminated with *B. cereus* spores at levels ranging from 3 to 100 per g (Table 1). A few (13%) contained >100 spores per g. Some brands of packaged seeds were exceptionally clean and were negative for aerobic sporeformers, as determined by culturing in Trypticase soy broth. Mung beans were contaminated more frequently and usually at a higher level than either alfalfa or wheat (Table 1). All types, however, were contaminated frequently enough to be potentially hazardous whenever conditions were suitable for rapid growth of *B. cereus*.

Growth from contaminated seeds

Data on the incidence and numbers of *B. cereus* found on sprouts grown from naturally contaminated seeds show that alfalfa and mung beans are relatively poor substrates for natural propagation of *B. cereus* during sprouting (Table 2). The numbers of this pathogen increased dramatically on wheat, however, and reached a level ($>10^5$/g) considered likely to cause food poisoning in 16 of the 25 (58%) wheat units examined. Such levels were not found with any of the 20 alfalfa units and only 3 of 20 mung bean units. These results indicate that although mung beans and alfalfa seeds are frequently contaminated with *B. cereus* spores, they are apparently relatively poor substrates for growth of this bacterium and thus present only a minimal hazard. These findings may explain the infrequent occurrence of *B. cereus* food poisoning from consumption of raw vegetable sprouts because alfalfa and mung bean sprouts are consumed more frequently than any other type. A definite hazard exists with sprouted wheat, however. Large populations of *B. cereus* were usually found on wheat sprouts—even those produced from seeds in which contamination was undetectable before sprouting.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/50/1/62/1656061/0362-028x-50_1_62.pdf by guest on 28 September 2022

TABLE 1. *Incidence of* B. cereus *in unsprouted vegetable seeds.*

Type of seeds	No. of test units	No. (%) of test units within population range[a]		
		<3	3-100	>100
Alfalfa	24	10(42)	9(38)	4(17)
Mung beans	40	7(40)	28(70)	5(13)
Wheat	24	11(46)	13(54)	2(8)
Mixtures	10	2(20)	6(60)	2(20)
Total	98	30(31)	56(57)	13(13)

[a]Determined by 3-tube MPN method on 1 g of seeds in T-soy polymyxin broth.

TABLE 2. *Growth of* B. cereus *on vegetable sprouts grown from naturally contaminated seeds.*

Type of sprouts	No. of test units	No. (%) of test units with *B. cereus* counts range[a]					Overall mean
		$<10^3$	10^3-10^4	10^4-10^5	10^5-10^6	$>10^6$	$\log_{10}$/g
Alfalfa	20	12(60)	5(25)	3(15)	0	0	3.72
Mung beans	20	9(45)	3(15)	5(25)	3(15)	0	4.52
Wheat	25	3(12)	2(8)	4(16)	9(36)	7(28)	5.39

[a]Determined by plate counts of 1 g homogenized sprouts on MYP agar.

Inoculated seeds

The number of *B. cereus* ($\log_{10}$/g) found on sprouts grown from mung bean, alfalfa and wheat seeds inoculated with moderate levels of spores of ten different food poisoning strains of *B. cereus* are shown in Table 3. As with naturally contaminated seeds, alfalfa and mung beans were relatively poor substrates for growth of *B. cereus,* whereas wheat supported rapid growth of all strains. Plate counts of $\geq 10^6$/g were found with eight of the ten strains of wheat sprouts, indicating that sprouted wheat is potentially very hazardous when grown from contaminated seeds. This hazard is further compounded by the fact that washing and cooking wheat sprouts is minimally effective in reducing *B. cereus* contamination.

Effect of washing and cooking

The effects of washing and cooking on the numbers of *B. cereus* found on mung bean and wheat sprouts are shown in Table 4. Washing sprouts three times resulted in approximately one log unit reduction in counts of *B. cereus* on mung bean sprouts. Heating bean sprouts for 20 min at 95°C to simulate cooking reduced contamination to insignificant levels ($<10^4$/g) for all five strains. Washing wheat sprouts, however, was less effective because growth apparently had occurred within the wheat kernel and abundant root hairs on wheat sprouts made the washing procedure less effective. Heating wheat sprouts to simulate cooking was also ineffective in reducing the *B. cereus* counts to safe levels. This indicates that a large proportion of the viable *B. cereus* cells on wheat sprouts were spores.

There is presently no information available about the possible risk from consuming foods contaminated with large numbers of *B. cereus* spores. However, it seems likely that in the case of emetic strains, preformed toxin could survive heating for as long as 1 h at 100°C and still cause illness (*5,6*). Studies are presently under way in our laboratory and elsewhere to elucidate the basic pathogenic mechanisms of both diarrheagenic and emetic strains.

REFERENCES

1. Andrews, W. H., P. B. Mislivec, C. R. Wilson, V. R. Bruce, P. L. Poelma, G. Gibson, M. W. Trucksess, and K. Young. 1982. Microbial hazards associated with bean sprouting. J. Assoc. Off. Anal. Chem. 65:241-248.
2. Danielson, M. L., and T. K. Holm. 1983. Bacteriological quality of sprouts and salads containing sprouts. Var Foeda (Sweden) 35:11-19.
3. Harmon, S. M. 1982. New method for differentiating members of the *Bacillus cereus* group: collaborative study. J. Assoc. Off. Anal. Chem. 65:1134-1139.
4. Lancette, G. A., and S. M. Harmon. 1980. Enumeration and confirmation of *Bacillus cereus* in foods: collaborative study. J. Assoc. Off. Anal. Chem. 63:581-586.

TABLE 3. *Growth of* B. cereus *on vegetable sprouts grown from seeds inoculated with spores of* B. cereus.[a]

Seeds inoculated with strain[b]	Log of plate count/g on MYP agar[c]		
	Mung bean sprouts	Alfalfa sprouts	Wheat sprouts
B4ac	4.28[d]	4.85	6.15
F4433	5.26	3.11	6.36
F4810	5.32	4.30	6.51
F4165	5.58	5.00	6.59
F4552	4.76	4.80	5.43
19668	4.99	5.36	6.11
30020	5.70	4.54	7.04
F840	5.76	3.95	6.56
MB31	5.77	5.30	5.75
MB34	4.11	5.11	6.79

[a]Mung beans and alfalfa: 500 spores/g; wheat: 200 spores/g.
[b]Strains from food poisoning episodes.
[c]Five isolates from each test unit were confirmed as *B. cereus* by the method of Harmon (*3*).
[d]Average of duplicate determinations.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/50/1/62/1656061/0362-028x-50_1_62.pdf by guest on 28 September 2022

TABLE 4. *Effect of washing and heating on populations of* B. cereus *on mung bean and wheat sprouts.*

Type of sprouts	Seeds inoculated with strain[a]	Log_{10} of plate count/g on MYP agar[b]		
		Before treatment	After washing[c]	After heating[d]
Mung beans				
	F4433	5.44[e]	4.76	3.00
	F4165	6.04	5.04	3.95
	19668	5.39	3.38	3.00
	MB31	5.47	4.90	3.00
	MB34	6.25	5.45	3.00
Wheat				
	B4ac	5.51	4.79	4.30
	F4552	6.43	5.90	5.86
	F2769	5.88	5.76	3.60
	F840	6.11	5.75	4.36
	30020	6.57	6.30	5.43

[a]Before sprouting, seeds were inoculated with 200-500 *B. cereus* spores/g.
[b]Confirmed as *B. cereus* by AOAC method.
[c]Washed 5 min with a spray of cold water.
[d]Heated for 20 min at 95°C.
[e]Average of duplicate determinations.

5. Melling, J., and B. J. Capel. 1978. Characteristics of *Bacillus cereus* emetic toxin. FEMS Microbiol. Lett. 4:133-135.
6. Melling, J., B. J. Capel, P. C. B. Turnbull, and R. J. Gilbert. 1976. Identification of a novel enterotoxigenic activity associated with *Bacillus cereus*. J. Clin. Pathol. 29:938-940.
7. Portnoy, B. L., J. M. Goepfert, and S. M. Harmon. 1973. An outbreak of *Bacillus cereus* food poisoning resulting from contaminated vegetable sprouts. Am. J. Epidemiol. 103:589-594.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/50/1/62/1656061/0362-028x-50_1_62.pdf by guest on 28 September 2022