

BACILLUS

LETHALITY OF CHLORINE, CHLORINE DIOXIDE, AND A COMMERCIAL FRUIT AND VEGETABLE SANITIZER TO VEGETATIVE CELLS AND SPORES OF *BACILLUS CEREUS* AND SPORES OF *BACILLUS THURINGIENSIS* (L. R. Beuchat, C. A. Pettigrew, M. E. Tremblay, B. J. Roselle, and A. J. Scouten)

Concerns about international bioterrorism have rekindled an interest in developing and refining technologies to kill *Bacillus anthracis* spores in urban environments and in foods. While spores of several *Bacillus* species known to cause spoilage of foods and foodborne disease have been studied extensively to determine conditions affecting growth and sporulation, as well as their sensitivity to physical treatments and sanitizers, comparatively little is known about conditions affecting survival and growth of *B. anthracis* in foods and the effectiveness of sanitizers in killing spores of the organism on food-contact surfaces and in foods. *Bacillus anthracis* is closely related to *Bacillus cereus* and *Bacillus thuringiensis*, the principle distinguishing difference being the presence of virulence genes on plasmids in *B. anthracis*. Direct comparisons of the sensitivity of spores of *B. anthracis* and spores of other *Bacillus* species to sanitizers used to decontaminate food-contact surfaces and foods have not been described. Information on the sporicidal activity of chemical treatments using *B. cereus*, *B. thuringiensis*, and perhaps other *Bacillus* species as potential surrogates for *B. anthracis* would provide insights to the relative sensitivity of *B. anthracis* spores to the same treatments.

We conducted a series of experiments to determine the effectiveness of chlorine, ClO₂, and a commercial raw fruit and vegetable sanitizer in killing vegetative cells and spores of *B. cereus* and *B. thuringiensis*. The goal is to eventually test the sensitivity of vegetative cells and spores of *B. anthracis* to treatments causing the highest reductions in populations of these potential surrogates. Insights to the sensitivity of *B. cereus* and *B. thuringiensis* to these sanitizers will be valuable in achieving that goal. Treatment with alkaline (pH 10.5 - 11.0) ClO₂ (200 µg/ml) produced by electrochemical technologies reduced populations of a five-strain mixture of vegetative cells and a five-strain mixture of spores of *B. cereus* by >5.4 and > 6.4 log₁₀ cfu/ml, respectively, within 5 min. This compares to respective reductions of 4.5 and 1.8 log₁₀ cfu/ml resulting from treatment with 200 µg/ml chlorine. Treatment with a 1.5% acidified (pH 3.0) solution of Fit[®] powder product (FPP) was less effective, causing 2.5 and 0.4 log₁₀ cfu/ml reductions in the number of *B. cereus* cells and spores, respectively. Treatment with alkaline ClO₂ (85 µg/ml), acidified (pH 3.4) ClO₂ (85 µg/ml), and a mixture of ClO₂ (85 µg/ml) and FPP (0.5%) (pH 3.5) caused reductions in vegetative cell/spore populations of >5.3/5.6, >5.3/5.7, and >5.3/6.0 log₁₀ cfu/ml, respectively. Treatment of *B. cereus* and *B. thuringiensis* spores in a medium (3.4 mg of organic and inorganic solids/ml) in which cells had grown and produced spores with an equal volume of alkaline (pH 12.1) ClO₂ (400 µg/ml) for 30 min reduced populations by 4.6 and 5.2 log₁₀ cfu/ml, respectively, indicating high lethality in the presence of materials other than spores that would potentially react with and neutralize the sporicidal activity of ClO₂.