Journal of Food Protection 89 (2026) 100677

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Isolation and Characterization of *Bacillus cereus* Group Species in Powdered Infant Formula and Infant Cereal Using a Newly Developed Detection System

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ARTICLE INFO

Keywords:
Bacillus cereus group
Enrichment method
Infant cereal
Powdered infant formula

ABSTRACT

Bacillus cereus group species are frequently detected at low levels in infant foods, including powdered infant formula and infant cereals. Although the infectious dose in neonates and infants has not been established, evidence suggests it may be approximately 1,000 CFU/g. When infant foods are exposed to temperature abuse, *B. cereus* group species could grow beyond acceptable limits, resulting in microbiologically unsafe products. Therefore, a detection scheme using an enrichment broth may be necessary to assess their potential risk in these critical foods. In this study, the R & F® B*acillus cereus* Group Enrichment Broth was developed, and a detection scheme was evaluated for its efficacy in recovering low-level *B. cereus* group species from powdered infant formula and infant cereals. Additionally, we used whole genome sequencing to gain deeper insights into the genomic characteristics of the recovered isolates and performed in vitro cytotoxicity assays to assess their virulence potential. The detection scheme recovered *B. cereus* group species in 52% (26/50) of samples that had initially screened below 100 CFU/g, resulting in a predictive positive value (PPV) of 97.6% as confirmed by whole-genome sequencing of the recovered isolates. Cytotoxicity testing of selected isolates revealed varying levels of toxicity toward Caco-2 cells. The results demonstrate that the enrichment method effectively recovered potentially virulent *B. cereus* group strains, highlighting the need for regular monitoring of these species in infant foods, especially for vulnerable populations such as premature neonates and infants.

The *Bacillus cereus* group is a diverse group of Gram-positive, spore-forming bacilli that are found widespread in the environment, with some species within this group associated with foodborne illness (Guinebretière et al., 2008). Notable species with pathogenic potential include *B. cereus sensu stricto* (*s.s.*), *B. anthracis*, *B. thuringiensis*, and *B. cytotoxicus* (Carroll et al., 2022; Carroll, Wiedmann, et al., 2020). Some strains of the *B. cereus* group produce cereulide, a heat-stable peptide causing emetic illness, as well as enterotoxins contributing to diarrheal illness (Ehling-Schulz et al., 2006). Among the enterotoxins, hemolysin BL (Hbl), nonhemolytic enterotoxin (Nhe), and cytotoxin K (CytK) have been linked to necrotic enteritis in healthy adults (Girisch et al., 2003; Lotte et al., 2022; Lund et al., 2000). Although the *B. cereus* group is not commonly recognized as a major pathogen associated with infant food products (World Health Organization, 2004, 2006), these microorganisms are of particular concern in the context of food contamination due to their heat-resistant endospores, which can withstand food manufacturing processes and remain viable throughout the shelf life of the product (Choi & Kim, 2020). These characteristics are especially relevant to infant foods, given that powdered infant formula and infant cereal are nonsterile and can naturally contain *B. cereus* group species (Adimpong et al., 2012; Pei et al., 2018; Rahimi et al., 2013; Sadek et al., 2018). In 2022, a study detected *B. cereus* group species harboring toxin genes in 26.7% of powdered infant formula and 36.7% of milk-cereal−based infant formula (Ibrahim et al., 2022), indicating that *B. cereus* group species are quite prevalent in infant foods. Multiple case studies highlight the severity of *B. cereus* infections in neonates, which can, in some cases, lead to death (Hilliard et al., 2003; Lotte et al., 2017; Machado et al., 2014; Patrick et al., 1989; Ramarao et al., 2014), suggesting a broader pathogenic potential in this vulnerable population than previously recognized. Moreover, a recent global meta-analysis of *B. cereus* infection reported a mortality rate of 0.9% and found that younger populations, including infants, tended to experience more severe symptoms of acute liver failure and multiorgan dysfunction, which raises concerns about the potential role of the *B. cereus* group species in infant food and related intestinal diseases (Li et al., 2025).

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https://doi.org/10.1016/j.jfp.2025.100677
Received 23 September 2025; Accepted 27 November 2025
Available online 01 December 2025