

Characterization and biocontrol of *Bacillus cereus* from food and environment

マルワ ナビル サエド アブデラジズ

<https://hdl.handle.net/2324/7396296>

出版情報 : Kyushu University, 2025, 博士 (農学), 課程博士
バージョン :
権利関係 :



Name : マルワ ナビル サエド アブデラジズ (Marwa Nabil Sayed Abdelaziz)

Title : Characterization and biocontrol of *Bacillus cereus* from food and environment
(食品及び環境由来 *Bacillus cereus* の特徴とバイオコントロール)

Category : Kou

Thesis Summary

Bacillus cereus sensu stricto is a Gram-positive, spore-forming, motile bacterium commonly found in soil, water, and various food products such as rice, vegetables, and dairy. It is a foodborne pathogen responsible for emetic and diarrheal syndromes due to toxins encoded by virulence genes, including *nheABC*, *entFM*, and *cesA*. In addition to causing food poisoning, it can lead to serious infections such as bacteremia and endophthalmitis. The widespread use of antibiotics has promoted the rise of antimicrobial-resistant (AMR) *B. cereus*, presenting a great threat to public health. In recent years, bacteriophages have emerged as a promising alternative tool to antibiotics for controlling pathogenic bacteria due to their specificity toward their bacterial host and safety to human. Therefore, this study aimed to investigate the prevalence, virulence, AMR profiles, and genetic diversity of *B. cereus* s. s. isolated from food and environmental sources in Fukuoka, Japan, and to explore biocontrol strategies using bacteriophage and other natural agents like nisin.

Firstly, a total of 179 samples were collected from food and environmental sources, and *B. cereus* s. s. was detected in 22.3% of them. All isolates exhibited psychrophilic growth, biofilm-forming ability, and carried at least one toxin gene. The most common virulence gene among them was *entFM* (57.5%), while the emetic toxin gene *cesA* was also detected in 17.5% of isolates. High diversity was revealed by Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry and multilocus sequence typing (MLST). According to MLST analysis, 23 novel sequence types and three new alleles were identified. Antibiotic resistance profiling showed an especially high resistance rate against tetracycline (42.5%), erythromycin (37.5%), and vancomycin (37.5%). All the isolates were resistant to seven or more antimicrobials, indicating high multidrug resistance. This underscores the developing issue of treating *B. cereus* infections.

Secondly, out of ten different food and environmental samples, six bacteriophages that target toxin-producing and multidrug-resistant *B. cereus* s. s. were isolated using the double-layer agar method. Among them, phage vB_BceS-M2 exhibited the broadest host range and the most potent lytic activity. Characterization of the phage demonstrated high stability across a wide range of pH levels and temperatures, supporting its suitability for food applications. Transmission electron microscopy showed that phage vB_BceS-M2 has an isometric head approximately 60 nm wide and a long, non-contractile tail about 170 nm long, which is typical of the siphovirus morphotype. Based on the latest classification by the International Committee on Taxonomy of Viruses, it is grouped under the class *Caudoviricetes*, which includes all tailed phages. One-step growth curve analysis revealed a short latent period of 20 min, followed by a rapid increase in phage titer, with a burst size of approximately 613 Plaque-Forming Unit per infected cell. Whole-genome sequencing of the phage indicated a circular double-stranded DNA genome of 56,482 bp and confirmed the absence of genes related to tRNA, virulence factors, or antimicrobial resistance, while showing the presence of an endolysin gene. These findings suggest that phage vB_BceS-M2 can be a safe and promising biocontrol agent against *B. cereus* s. s. in food.

Thirdly, the synergy between phage vB_BceS-M2 and nisin, a commonly used natural antimicrobial, was assessed using the checkerboard assay, which demonstrated a strong synergistic effect. In broth, phage vB_BceS-M2 significantly reduced viable counts of *B. cereus* s. s., and its combination with nisin exhibited a synergistic effect as it reduced the viable counts and delayed the regrowth of phage-resistant mutants at both 4 and 25°C. The synergistic antibacterial effects of phage vB_BceS-M2 and nisin against *B. cereus* s. s. were demonstrated in boiled rice, cheese, and meatballs at both refrigeration and room temperatures, but not in whole milk.

These findings underscore the efficacy of combining bacteriophages with natural antimicrobials like nisin in developing safe and effective biocontrol strategies against toxigenic and antibiotic-resistant *B. cereus* s. s. in diverse food matrices.