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Detection and Disinfection of Bacillus Cereus

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Abstract: Bacillus cereus is a foodborne, conditional pathogen that causes vomiting and diarrhea and has become a rising threat to food safety. This review introduces several major types of B. cereus detection methods (including traditional methods, PCR-based assays , immunological assays, cytotoxicity assays, and a CBD-related assay) and species-specific disinfection methods (including AMPs and endolysins). These methods have either been applied or have the potential to be applied in the food industry. The intention of this review is to introduce the principle of these methods and evaluate their strengths and weakness.

Keywords: Bacillus cereus; Bacteria detection; Disinfection; Food safety

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1. Introduction

Bacillus cereus is a type of gram-positive bacteria that naturally exists in the environment [1]. However, it is also widely present in protein or carbohydrate-rich food even in developed countries that have relatively better food processing conditions [2]. Although only some B. cereus strains are pathogenic, these strains would cause vomiting (related to contaminated starchy food) and diarrhea (related to contaminated vegetables and meat) [3] when present in the human digestive tract and cause endophthalmitis, bacteremia, and other types of inflammations if present in blood vessels. Various strains of B. cereus could secrete four types of hemolysins, three types of distinct phospholipases, and three types of pore-forming enterotoxins [4]. Moreover, they could produce endospores that are extremely heat resistant and cannot be destroyed by conventional cooking methods [3].

Although B. cereus could be killed by heating with pressure [3] or most broad-spectrum antibiotics (except β-lactam antibiotics) [5], these methods might not be practical in the fermented foods and desserts industry since re-heating may destroy their products' flavor, and broad-spectrum antibiotics will kill non-pathogenic strains used in fermentation. Methods of detecting and eliminating B. cereus, as well as treating related diseases are required for these industries.

2. Current detection methods for B. Cereus

2.1. Traditional method

The harm of B. cereus infection had long been disregarded due to its widespread in nature [6]. Thus, accurate detection of B. cereus is vital in terms of improving food safety. However, the structural and genetic similarity of B. cereus and other strains in the B. cereus group hinder the accuracy of the detection [7]. The traditional method of identifying B. cereus colonies is to culture B. cereus in food sample extraction on agar plates and perform morphology and biochemical assays [8]. Additionally, chromogenic Bacillus cereus Agar Plate could assist in the identification of colonies since the metabolic activity of B. cereus changes

the color of the culture medium [9]. Although these tests are very accurate, it is also time-consuming and the materials, instruments, and professional technicians are usually unavailable for medium to small scale food manufacturing companies.

2.2. Polymerase chain reaction- based (PCR) assays

Many toxin-related genes such as bceT (coding for enterotoxin T) [10] , hblC, hblD, hblA, hblB (coding for components of hemolysin BL) [11], ces (coding for cereulide) [12], and nheA, nheB nheC (coding for components of non-hemolytic Enterotoxin) [13] have been identified, thus allowing PCR to be a method to detect B. cereus strains [10]. However, a strain with toxin-encoding genes does not necessarily mean it could secrete toxins due to the complex regulative mechanisms of the B. cereus group's toxins and the health condition of hosts [14]. Also, various types of toxin genes among different strains in the B. cereus group make PCR that targets a single toxin-related gene insufficient to exclude all possible toxins that are present [15]. Advanced amplification and electrophoresis methods such as rep-PCR, Random Amplified Polymorphic DNA (RAPD)-PCR and PCR-TTGE could be a solution, but these methods still cannot distinguish between B. cereus and B. thuringiensis due to their extremely high genetic similarity and will cost longer time than normal PCR [16].

2.3. Immunological assays

To accurately detect toxic strains, immunological assays that target B. cereus toxins could be applied. Mouse monoclonal antibodies and rabbit antiserum that target every 3 components of hemolysin BL (HBL) and every 3 components of non-hemolytic Enterotoxin (Nhe), which both types of toxins are diarrheal-related three-component enterotoxins from B. cereus, are well developed and characterized [17,18]. Besides, rabbit antisera and mouse monoclonal antibodies that target the N-terminal of B. cereus flagellin protein are recently being developed and were proven to be powerful tools in detecting B. cereus [19]. However, cerulide, the emetic toxin of B. cereus, is not antigenic and cannot be detected through immunological assays [20]. Still, the cost of antibodies and testing devices are not affordable for developing areas.

2.4. Cytotoxicity assays

Another category of toxin assay, including water-soluble tetrazolium salt (WST-1)-based assay and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT)-based assay, utilizes B. cereus toxins' cytotoxicity to survive in mammalian cells. B. cereus culture supernatants are added to the Chinese hamster ovary (CHO) cell line culture (other mammalian cell lines might also be acceptable) and the cell's metabolic product is measured. To be more specific, reductase in living cells' mitochondria could reduce MTT to insoluble Formazan [21] or reduce WST-1 to soluble Formazan [22]. while cells killed by B. cereus toxin could not. Both types of Formazan have specific absorbance wavelengths, thus a functional relationship could be established through cytotoxicity, living cell proportion, and Formazan's absorbance. Although the principle of the two assays are the same, the MTT assay needs a minimum of 44–52 hours to complete due to the long dissolving process of Formazan, while WST-1 assay only needs 3 hours to complete [22]. It is also noteworthy that the duration of both assays does not include the time of culturing and identifying B. cereus group strains, and only samples from bacteria culture are acceptable for the assays.

2.5. Cell-binding domain (CBD)- conjugated magnetic nanoparticles detection method

A more convenient method of detection involves cell-binding domain (CBD)-conjugated magnetic nanoparticles. Cell-binding domain is a component of endolysin (the chemical that bacteriophages secrete to lyse the cell) that could specifically bind to a species of bacteria's cell wall. Thus the CBD-conjugated magnetic nanoparticles serves could bind and mark B. cereus, which could later be separated by a magnet.

If B. cereus is present in the separated sample, an ATP luminance assay could detect the ATP in the bacteria and show its presence. This method is more affordable and time-efficient but has a higher detection limit which is 103-104 cells [23].

3. Specific disinfection methods for B. cereus

3.1. Antimicrobial peptides

Fermented beans or rice are common foods in Asian countries, but they are also very likely to be contaminated by B. cereus during the fermentation process [24]. To prevent such contamination, Korean scientists isolated several types of antimicrobial peptides (AMP), proteins that kill competing bacterias, that target B. cereus from B. subtilis. These AMPs include UV254-B which has antimicrobial activity on both B. cereus and Listeria monocytogenes [25], IC-1 that targeted specifically on B. cereus [26], an AMP from B. Subtilis HJ18-4 that could both inhibit B. cereus growth and expression of toxin [27], and many other AMPs that have similar properties. Since B. Subtilis are non-pathogenic bacteria that are normally used during food fermentation, applying these AMPs in the food industry is an easy way to eliminate B. cereus contamination.

AMPs are also potential medicines for treating bacterial infections. For example, Mersacidin, another AMP from B. subtilis that targets staphylococcus aureus, shows a similar antimicrobial activity as commercial antibiotics [28]. However, most genes that code B. cereus targeted AMPs have not been sequenced and their related study mainly focused on protein composition and structure, bactericidal kinetics, and bactericidal mechanisms [29]. This is probably because most of these AMPs are expressed by wild-isolated strains that have unknown genome sequences, and the food producer just needs to add the strain rather than proteins during fermentation. Thus, more research is required to turn B. cereus target AMPs into medicines that can be massively produced by model organisms.

3.2. Endolysins

Compared to AMPs, endolysins are better studied, could be easier expressed, and has greater potential to be medicines that treat B. cereus. As discussed previously, they are two-domain proteins that have a cell-binding domain (CBD) that specifically recognizes and binds to bacteria's cell wall and an enzymatically active domain that break specific cell wall structure and lyse the cell [30]. Since endolysins originated from bacteriophages, they are usually smaller in size and their corresponding gene is easier to be located and sequence [31]. Their simpler structure allows their mechanism to be thoroughly studied and to be correctly folded in E. coli, which further allows them to be massively produced. Although endolysin could be an antigen, it usually does not trigger an immune response, making them considerably safe when being applied to the human body [32]. The only possible drawback of endolysin is that its CBD may bind to all types of bacteria with similar cell wall structures, making it inaccurate when eliminating one specific type of bacteria [33]. However, researchers have already found endolysin LysPBC5 that has a very narrow lysis spectrum that only contains B. cereus [29]. Also, since numerous types of bacteriophages host on B. cereus, the fusion of a specific CBD and a specific EAD could further enhance the specificity of fused endolysin and provide a solution to the problem [34].

Conclusion

In the review, several types of detection methods for B. cereus were discussed. Each method has its own advantage in accuracy, efficiency, or cost. Overall, the CBD conjugated magnetic nanoparticles detection method has the greatest advantage in efficiency, but it still needs a lower detection limit. All methods described require professional laboratory conditions, and are not fast enough to test the food sample that is

going to be served. Thus, they are not practical in small catering enterprises, and a more convenient and efficient way has to be developed to ensure food safety.

For disinfection methods, AMPs are commonly used in the fermentation industry but are harder to be applied in other fields, whereas endolysins have several advantages and have greater potential to be medicines that treat B. cereus infection. With technical improvement, B. cereus infection would definitely less harm to humans.

Disclosure statement

The author declares no conflict of interest.

References

[1] Savini, V, 2016, Chapter 6- Bacillus cereus Pneumonia, in Diverse Faces of Bacillus Cereus ScienceDirect: Academic Press, 73–84. https://doi.org/10.1016/B978-0-12-801474-5.00006-2

[2] Berthold-Pluta A, Pluta A, Garbowska M, et al., 2019, Prevalence and Toxicity Characterization of Bacillus Cereus in Food Products from Poland. Foods, 8(7): 269. https://doi.org/10.3390/foods8070269

[3] Schneider KR, Parish ME, Goodrich RM, et al., 2005, Preventing Foodborne Illness: Bacillus Cereus and Bacillus Anthracis. EDIS, 2005(1): FSHN04-05. https://doi.org/10.32473/edis-fs103-2004

[4] McDowell RH, Sands EH, & Friedman H, 2022, Bacillus Cereus, StatPearls Publishing, Florida. https://www.ncbi.nlm.nih.gov/books/NBK459121/#:~:text=Bacillus%20cereus%20is%20a%20toxin-producing%20facultatively%20anaerobic%20gram-positive

[5] Fiedler G, Schneider C, Igbinosa EO, et al., 2019, Antibiotics Resistance and Toxin Profiles of Bacillus Cereus-Group Isolates from Fresh Vegetables from German Retail Markets. BMC Microbiology, 19(1):1–13. https://doi.org/10.1186/s12866-019-1632-2

[6] Glasset B, Herbin S, Granier SA, 2018, Bacillus Cereus, A Serious Cause of Nosocomial Infections: Epidemiologic and Genetic Survey. PLOS ONE, 13(5): e0194346. https://doi.org/10.1371/journal.pone.0194346

[7] Ramarao N, Tran S-L, Marin M, et al., 2020, Advanced Methods for Detection of Bacillus Cereus and Its Pathogenic Factors. Sensors, 20(9):2667. https://doi.org/10.3390/s20092667

[8] Bacterial Analytical Manual, 2012, BAM Chapter 14: Bacillus Cereus, amended October 2020, US Food & Drug Administration, viewed July 25, 2022. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-14-bacillus-cereus

[9] Pontieri E, 2016, Bacillus Cereus Group Diagnostics, in The Diverse Faces of Bacillus Cereus, 15–33. https://doi.org/10.1016/b978-0-12-801474-5.00002-5

[10] Hansen BM, & Hendriksen NB, 2001, Detection of Enterotoxic Bacillus Cereus and Bacillus Thuringiensis Strains by PCR Analysis. Applied and Environmental Microbiology, 67(1): 185–189. https://doi.org/10.1128/aem.67.1.185-189.2001

[11] Heinrichs JH, Beecher DJ, MacMillan, JD, et al, 1993, Molecular Cloning and Characterization of the Hbla Gene Encoding the B Component of Hemolysin BL From Bacillus Cereus. Journal of Bacteriology, 175(21): 6760–6766. https://doi.org/10.1128/jb.175.21.6760-6766.1993

[12] Kubota N, Kobayashi J, Kasai A, et al., 2022, Detection of Bacillus Cereus as a Causative Agent of Emetic Food Poisoning by an Unconventional Culture Procedure. Journal of Infection and Chemotherapy, 2022: 35870790. https://doi.org/10.1016/j.jiac.2022.07.011

[13] Granum PE, O'sullivan K, Lund T, 1999, The Sequence of the Non-haemolytic Enterotoxin Operon From Bacillus Cereus. FEMS Microbiology Letters, 177(2): 225–229. https://doi.org/10.1111/j.1574-6968.1999.tb13736.x

[14] JeÃŸberger N, Krey VM, Rademacher C, et al., 2015, From Genome to Toxicity: A Combinatory Approach Highlights the Complexity of Enterotoxin Production in Bacillus Cereus. Frontiers in Microbiology, 6: 560. https://doi.org/10.3389/fmicb.2015.00560

[15] Cardazzo B, Negrisolo E, Carraro L, et al., 2008, Multiple-Locus Sequence Typing and Analysis of Toxin Genes in Bacillus cereus Food-Borne Isolates. Applied and Environmental Microbiology, 74(3): 850–860. https://doi.org/10.1128/aem.01495-07

[16] Manzano M, Giusto C, Iacumin L, 2009, Molecular Methods to Evaluate Biodiversity in Bacillus Cereus and Bacillus Thuringiensis Strains from Different Origins. Food Microbiology, 26(3): 259–264. https://doi.org/10.1016/j.fm.2008.12.012

[17] Dietrich R, Fella C, Strich S, et al., 1999, Production and Characterization of Monoclonal Antibodies Against the Hemolysin BL Enterotoxin Complex Produced by Bacillus Cereus. Applied and Environmental Microbiology, 65(10): 4470–4474. https://doi.org/10.1128/aem.65.10.4470-4474.1999

[18] Dietrich R, Moravek M, Bürk C, 2005, Production and Characterization of Antibodies Against Each of the Three Subunits of the Bacillus Cereus Nonhemolytic Enterotoxin Complex. Applied and Environmental Microbiology, 71(12): 8214–8220. https://doi.org/10.1128/aem.71.12.8214-8220.2005

[19] Schwenk V, Dietrich R, Klingl A, 2022, Characterization of Strain-Specific Bacillus Cereus Swimming Motility and Flagella by Means of Specific Antibodies. PLOS ONE, 17(3): e0265425. https://doi.org/10.1371/journal.pone.0265425

[20] Granum PE, Lund T, 2006, Bacillus Cereus and its Food Poisoning Toxins. FEMS Microbiology Letters, 157(2), 223–228. https://doi.org/10.1111/j.1574-6968.1997.tb12776.x

[21] Mosmann T, 1983, Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. Journal of Immunological Methods, 65(1-2): 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

[22] Ngamwongsatit P, Banada PP, Panbangred W, 2008, WST-1-based Cell Cytotoxicity Assay As A Substitute for MTT-Based Assay for Rapid Detection of Toxigenic Bacillus Species Using CHO Cell Line. Journal of Microbiological Methods, 73(3): 211–215. https://doi.org/10.1016/j.mimet.2008.03.002

[23] Park C, Kong M, Lee J-H, et al., 2018, Detection of Bacillus Cereus Using Bioluminescence Assay with Cell Wall-binding Domain Conjugated Magnetic Nanoparticles. BioChip Journal, 12(4): 287–293. https://doi.org/10.1007/s13206-018-2408-8

[24] Sanjukta S, Rai AK, 2016, Production of Bioactive Peptides During Soybean Fermentation and Their Potential Health Benefits. Trends in Food Science & Technology, 50: 1-10. https://doi.org/10.1016/j.tifs.2016.01.010

[25] Wu W-J, Park S-M, Ahn, B.-Y, 2013, Isolation and Characterization of An Antimicrobial Substance from Bacillus Subtilis BY08 Antagonistic to Bacillus Cereus And Listeria Monocytogenes. Food Science and Biotechnology, 22(2): 433–440. https://doi.org/10.1007/s10068-013-0098-5

[26] Yeo I.-C, Lee NK, Cha, C-J, 2011, Narrow Antagonistic Activity of Antimicrobial Peptide from Bacillus Subtilis SCK-2 Against Bacillus Cereus. Journal of Bioscience and Bioengineering, 112(4): 338–344. https://doi.org/10.1016/j.jbiosc.2011.06.011

[27] Eom JS, Lee, SY, Choi HS, 2014, Bacillus Subtilis HJ18-4 From Traditional Fermented Soybean Food

Inhibits Bacillus Cereus Growth and Toxin-Related Genes. Journal of Food Science, 79(11): M2279-M2287. https://doi.org/10.1111/1750-3841.12569

[28] Sumi CD, Yang BW, Yeo I.-C, et al., 2015. Antimicrobial Peptides of the Genus Bacillus: A New Era for Antibiotics. Canadian Journal of Microbiology, 61(2): 93-103. https://doi.org/10.1139/cjm-2014-0613

[29] Han J, Zhao S, Ma Z, Gao L, et al., 2017, The Antibacterial Activity and Modes of LI-F Type Antimicrobial Peptides Against Bacillus Cereus In Vitro. Journal of Applied Microbiology, 123(3): 602–614. https://doi.org/10.1111/jam.13526

[30] Lee KO, Kong M, Kim I, et al., 2019, Structural Basis for Cell-Wall Recognition by Bacteriophage PBC5 Endolysin. Structure, 27(9): 1355-1365.e4 https://doi.org/10.1016/j.str.2019.07.001

[31] Loessner MJ, Maier SK, Daubek-Puza H, et al., 1997. Three Bacillus Cereus Bacteriophage Endolysins Are Unrelated but Reveal High Homology to Cell Wall Hydrolases from Different Bacilli. Journal of Bacteriology, 179(9): 2845–2851. https://doi.org/10.1128/jb.179.9.2845-2851.1997

[32] Loeffler JM, Djurkovic S, Fischetti VA, 2003, Phage Lytic Enzyme Cpl-1 as a Novel Antimicrobial for Pneumococcal Bacteremia. Infection and Immunity, 71(11): 6199–6204. https://doi.org/10.1128/iai.71.11.6199-6204.2003

[33] Na H, Kong M, Ryu S, 2016, Characterization of LysPBC4, A Novel Bacillus Cereus-specific Endolysin of Bacteriophage PBC4. FEMS Microbiology Letters, 363(12): fnw092. https://doi.org/10.1093/femsle/fnw092

[34] Schmelcher M, Donovan DM, Loessner MJ, 2012, Bacteriophage Endolysins as Novel Antimicrobials. Future Microbiology, 7(10): 1147–1171. https://doi.org/10.2217/fmb.12.97