Evaluation of a Rapid Chromogenic Medium for the Quantitative Detection of *Bacillus cereus*

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Abstract [**Objectives**] To evaluate the performance of two rapid chromogenic media for the detection of *Bacillus cereus* in milk powder, and verify the media's inclusivity, exclusivity, and accuracy, and to assess their applicability for the quantitative detection of *B. cereus*. [**Methods**] *B. cereus* in milk powder samples was quantified using two rapid chromogenic media in combination with the national standard method. Agreement between the quantitative results from the three methods was subsequently assessed for agreement via a paired *t*-test. [**Results**] No significant differences were observed between the bacterial counts yielded by the two rapid chromogenic media and the national standard method ($P>0.05$), with excellent agreement between them. [**Conclusions**] The method of rapid chromogenic culture medium is rapid and simple.

Key words *Bacillus cereus*, Rapid, Chromogenic culture medium, Counting

0 Introduction

Since the early 20th century, The role of *B. cereus* as a cause of food poisoning has been well-established[1-2]. It is a common foodborne pathogen, ubiquitously found in soil, dust, water, and a wide range of food products[3-5]. This bacterium produces various toxins, including those causing diarrheal and emetic (vomiting) syndromes. Food poisoning incidents due to *B. cereus* can lead to symptoms such as nausea, vomiting, and diarrhea, posing a great threat to public health[6-9].

Dairy products are highly susceptible to contamination by *B. cereus* due to their rich content of proteins, fats, and other essential nutrients, combined with the multiple stages involved in their production, processing, storage, and transportation. Given the potential risk of bacterial growth in dairy products, establishing an accurate and efficient detection system is crucial to ensuring their quality and safety. While the traditional national standard method is highly accurate, its lengthy procedures and delayed results limit its applicability for rapid screening in the modern dairy industry. This limitation has spurred interest in rapid chromogenic media, an emerging detection technology. Its advantages, convenient operation, visual result interpretation, and rapid turnaround time, have made it a focus of recent research in food safety testing. A comparative framework was developed in this study to evaluate the performance of three media for quantifying *B. cereus*: the national standard medium and two novel rapid chromogenic media. The assessment was based on four core criteria: inclusivity (the ability to detect target bacteria), exclusivity (the capacity to suppress non-target bacteria), accuracy (the agreement between test results and reference methods), and operational efficiency (including ease of use and timeliness). A paired *t*-test was employed to conduct a horizontal comparison of three detection methods: the MYP (Mannitol Yolk Polymyxin) agar plate count method specified in the GB 4789.14-2014 national standard[10], and the rapid chromogenic medium methods from BIO-RAD and Biokar. The objective was to quantitatively analyze the significance of differences in their results for *B. cereus* in dairy samples. This study aims to provide a scientific and optimal detection solution for dairy producers, thereby enhancing quality and safety control throughout the entire chain from raw materials to finished products.

1 Materials and methods

1.1 Sample information The milk powder sample was purchased from JD.com.

1.2 Standard strain *Bacillus cereus* (CICC 21261), *Staphylococcus epidermidis* (CICC 10436), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922).

1.3 Instruments and Reagents Mannitol-Egg-Yolk-Polymyxin Agar Base and its supporting reagents (Beijing Land Bridge Technology Co., Ltd.), COMPASS® *Bacillus cereus* Agar and its supporting reagents (Biokar), RAPID' *Bacillus cereus* Agar and its supporting reagents (BIO-RAD), Brain Heart Infusion Agar Medium (Beijing Land Bridge Technology Co., Ltd.), Brain Heart Infusion Broth (Beijing Land Bridge Technology Co., Ltd.), Tryptone Soya Agar (Beijing Land Bridge Technology Co., Ltd.).

Biosafety cabinet; stomacher homogenizer; constant temperature incubator; biological microscope; fully automated microbial identification system.

1.4 Test methods

1.4.1 Inclusive and exclusive test. Standard strains: *B. cereus* CICC 21261, *B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212,

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S. epidermidis CICC 10436, *E. coli* ATCC 25922. The target strain was inoculated at a level of $10^2 - 10^3$ CFU using the following procedure: *B. cereus* was first streaked onto a Tryptic Soy Agar (TSA) plate for activation and cultured statically overnight in a constant-temperature incubator at 30 ℃ to restore bacterial activity. The next day, typical colonies were selected and adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using Brain Heart Infusion (BHI) broth. The suspension was then serially diluted with sterile saline to a concentration gradient of 10^{-5}, resulting in a final experimental bacterial suspension of 1.5×10^3 CFU/mL. A 1 mL aliquot of this suspension was inoculated into 225 mL of sterile saline to prepare the diluted sample solution. Synchronous detection was performed using the MYP agar plate count method specified in national standard GB 4789.14, along with two commercial *Bacillus cereus* rapid chromogenic medium methods. By comparing the colony morphology and color reaction of the target strain across the three media, the detection compatibility of *B. cereus* on different culture platforms was evaluated.

1.4.2 Accuracy test. Target strain: *B. cereus* CICC 21261. Target strain was inoculated at the level of: 0 (negative control), 1.5×10^2 CFU/g (Sample I). Inoculation procedure: The target *B. cereus* strain was streaked onto a TSA plate for activation and incubated overnight at 30 ℃ in a constant-temperature incubator until single colonies appeared. A single colony was then picked and transferred into 10 mL of BHI broth, followed by another overnight static incubation at 30 ℃. The resulting culture was serially diluted with sterile saline to a 10^{-3} concentration gradient. The bacterial concentration of this dilution was confirmed to be 1.5×10^3 CFU/mL using the plate counting method.

1.4.3 National standard method. According to the plate count method specified in Method 1 of national standard GB 4789.14-2014[10], 10 parallel tests were conducted for Sample I. First, 25 g of Sample I was weighed into a sterile homogenizing bag, and 225 mL of sterile physiological saline was added (1∶10, *v/v*) to prepare the initial homogenate. This was followed by a ten-fold serial dilution, with the 10^{-1} and 10^{-2} dilution levels selected for testing. From each dilution, three aliquots of 0.3, 0.3, and 0.4 mL were spread onto the surface of MYP agar plates. The inoculated plates were incubated upside down at (30 ± 1) ℃ for 48 h in a constant temperature incubator. After incubation, typical colonies were counted and identified.

1.4.4 *Bacillus cereus* rapid chromogenic medium method. *B. cereus* in Sample I was analyzed using a parallel detection method. A 25 g portion of the sample was weighed into a sterile homogenization bag, and 225 mL of sterile saline was added to prepare a 1∶10 homogenate. Two dilution levels (10^{-1} and 10^{-2}) were selected for testing. From each dilution, 1 mL was transferred into separate Petri dishes. Subsequently, COMPASS® *Bacillus cereus* Agar and RAPID *Bacillus cereus* Agar were poured into the respective plates. All plates were incubated at (30 ± 1) ℃ for 24 h in a constant-temperature incubator, after which typical colonies were enumerated.

1.4.5 Calculation method. A paired *t*-test was performed to compare the enumeration results of *B. cereus* in milk powder obtained by three different detection methods. The experiment was conducted as described previously, with each sample tested in ten replicates. Minitab software was used to analyze the paired *t*-test data and to determine whether there were significant differences among the mean values of the results from the three methods[11].

2 Results and analysis

2.1 Inclusive and exclusive test results The inclusivity of the target *B. cereus* standard strain was evaluated on MYP agar and two rapid chromogenic media. Typical colony morphology was observed on all three media, and the enumeration results were consistent with the theoretical spiking range, demonstrating good inclusivity (Table 1). The exclusivity was tested with non-target strains (*B. subtilis*, *E. faecalis*, *S. epidermidis*, and *E. coli*) on MYP and two chromogenic media. Key observations are as follows: *B. subtilis* on MYP agar produced yellow colonies without a halo, distinguishable from *B. cereus*, and was inhibited on the two chromogenic media. *E. faecalis* formed white colonies on COMPASS® *Bacillus cereus* Agar, contrasting with the blue-green *Bacillus cereus*, and was inhibited on the other two media. *S. epidermidis* and *E. coli* did not grow on any of the three media, with RAPID *B. cereus* Agar showing particularly effective inhibition. In summary, the rapid chromogenic media allowed for clear distinction between target and non-target bacteria, demonstrating good exclusivity (Table 2).

Table 1 Results of containment experiments of MYP and two rapid chromogenic medium methods

Culture medium name	Artificially contaminated samples	Spiked strain	Inoculation level // CFU/g	Theoretical range // CFU/g	*B. cereus* counting results // CFU/g
MYP	Sample I	*B. cereus* CICC 21261	800	444 – 1 440	860
COMPASS® *Bacillus cereus* Agar	Sample II		800	444 – 1 440	900
RAPID *Bacillus cereus* Agar	Sample III		800	444 – 1 440	1 000

Table 2 Exclusivity test results of MYP and two rapid chromogenic medium methods

Strain name	MYP	COMPASS® *Bacillus cereus* Agar	RAPID *Bacillus cereus* Agar
B. cereus CICC 21261	Pink colonies with halos	Blue-green colonies, 1 – 3 mm	Red colonies with opaque halos
B. subtilis ATCC 6633	Yellow colonies without halos	–	–
E. faecalis ATCC29212	–	White colonies	–
S. epidermidis CICC 10436	–	–	–
E. coli ATCC25922	–	–	–

2.2 Accuracy test results The presence of *Bacillus cereus* in Sample I was quantified using the national standard method and two rapid chromogenic culture methods (Table 3). The results, presented as the mean of two parallel experiments, were subjected to a paired *t*-test. We used Minitab software to perform the analysis, pairing the data from the national standard method with that from each rapid method separately, with a significance level (α) of 0.05. The results of these statistical comparisons are shown in Tables 4 and 5.

The data in Table 4 show that the mean values obtained from the national standard method, the Biokar rapid chromogenic medium method, and the BIO-RAD rapid chromogenic medium method were 175, 138, and 164 CFU/g, respectively, with corresponding standard deviations of 25.93, 18.38, and 18.97. A comparison of the standard deviations, Biokar rapid chromogenic medium method < BIO-RAD rapid chromogenic medium method < national standard method, indicates that, at the same sample concentration, the Biokar method yields results with relatively lower variability.

As shown in Table 5, the analysis of detection results between the national standard method and the Biokar rapid chromogenic medium method revealed a mean difference of 19, with a standard deviation of 33.1. The calculated *t*-value was 1.81 with 9 degrees of freedom, and the corresponding *P*-value was 0.103 (>0.05). According to statistical principles, this indicates no significant difference between the results obtained by the national standard method and the Biokar rapid chromogenic medium method. Similarly, analysis of the national standard method and the BIO-RAD rapid chromogenic medium method showed a mean difference of 11, with a standard deviation of the difference of 29.98, a *t*-value of 1.16, and 9 degrees of freedom. The *P*-value was 0.276 (>0.05), also indicating no significant difference between the two methods. In summary, compared with the national standard method, both the Biokar and BIO-RAD rapid chromogenic medium methods demonstrated better selectivity, more effectively preventing interference from miscellaneous bacteria, thereby resulting in relatively smaller systematic bias in the detection results.

Table 3 Count results of *Bacillus cereus* in sample I detected by three culture media

No.	National standard method	Biokar method	BIO-RAD method
1	130	150	150
2	180	170	170
3	140	140	140
4	180	160	160
5	190	140	170
6	190	150	160
7	180	140	150
8	160	180	190
9	220	140	150
10	180	190	200

Table 4 Basic descriptive statistics of test results of sample I

Method	Mean	N	Standard error	Standard error of the mean
National standard method	175	10	25.93	8.20
Biokar method	138	10	18.38	5.81
BIO-RAD method	164	10	18.97	6.00

Table 5 Pairwise statistical comparison and analysis results of sample I

Name	95% confidence interval of the difference							
	Mean	Standard error	Standard error of the mean	Lower limit	Upper limit	*t*	*df*	Sig (two-tailed)
National standard method—Biokar method	19	33.10	10.5	−4.70	42.70	1.81	9	0.103
National standard method—BIO-RAD method	11	29.98	9.48	−10.45	32.45	1.16	9	0.276

4 Conclusions and discussion

The results of this study demonstrated that when milk powder samples containing the same concentration of *Bacillus cereus* were tested using the national standard method, the Biokar rapid chromogenic medium method, and the BIO-RAD rapid chromogenic medium method for bacterial enumeration, the statistical analysis revealed no significant differences between the results obtained from the two rapid methods and those from the national standard method. This indicates that the test results of all three methods are in good agreement.

Based on the aforementioned results, both the Biokar and BIO-RAD rapid chromogenic medium methods are feasible for the quantitative detection of *B. cereus*. Compared with the MYP plate method specified in current Chinese food safety standards, the two rapid chromogenic medium methods exhibit superior specificity. Furthermore, the chromogenic plates are simple and convenient to operate, require less technical expertise from personnel, and allow detection to be completed merely by observing colony morphology and color, thereby significantly improving detection efficiency.

When quantifying *Bacillus cereus* in milk powder, both the MYP plate and the rapid chromogenic plate can achieve satisfactory detection results. However, the rapid chromogenic plate offers a faster detection process, with an incubation time shortened by approximately 24 h. Therefore, the rapid chromogenic plate can be recommended as a high-quality medium for the enumeration of *B. cereus* in milk powder.

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