**Comparative Evaluation of Selective Media for the Detection of Bacillus cereus**

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The commercially available 3 types of selective media in Japan were compared for the detection of *Bacillus cereus*. When assessed inclusivity using 25 *B. cereus* strains, MYP agar, NGKG agar, and chromogenic X-BC agar demonstrated excellent inclusivity. For exclusivity study using 50 non-*B. cereus* strains, MYP, NGKG, and X-BC allowed to grow 11, 7, and 3 strains, respectively. Of the grown bacteria on each strains tested, only 2 strains of *B. thuringiensis* formed typical *B. cereus* colonies on all selective media tested.

The NGKG and X-BC were compared with MYP as a reference using artificially contaminated food (fried rice, plain rice, fried noodle, and potato salad), since MYP is recommended in ISO 7932: 2004. The both correlation coefficients between NGKG and MYP, and X-BC and MYP were 0.999. Therefore, we demonstrated that NGKG and X-BC can be adapted to ISO 7932: 2004 method for selected food as well as MYP.

Key words: *Bacillus cereus* / Selective medium / Detection / ISO 7932: 2004.

*Bacillus cereus* has a ubiquitous habitation in the environment and has many opportunities to contaminate into food or the food processing environment (Bennett et al., 2015; Schoeni and Wong, 2005). Since *B. cereus* can produce emetic toxin and diarrheal toxins in addition to many kinds of protease, it can cause not only food spoilage and quality deterioration but also food poisoning via these toxins (Crielly et al., 1994; Fangio et al., 2010; Granum and Lund, 1997; Melling and Capel, 1978; Shinagawa et al., 1985; Vilas-Bôas et al, 2007). Hence, the control of *B. cereus* is necessary for both maintenance of food quality and prevention of food poisoning.

In Japan, the Mannitol egg yolk polymyxin (MYP) agar and Kim and Goepfert agar with NaCl and Glycine (NGKG) agar have been used well as commercially available selective agar media for the detection of *B. cereus* (Bennett et al., 2015; Fricker et al., 2008; Kim and Goepfert, 1971; Mossel et al., 1967). These media contains both phenol red and egg yolk for the differentiation of *B. cereus* as per the manufacturers’ information (Table 1), however the typical reaction of *B. cereus* colonies tends to diffuse and interfere with non-*B. cereus* colonies grown on these agar plates. Hence, these media need skills and experiences for differentiation of typical *B. cereus* colonies grown on these agar plates. The NGKG and X-BC agar (X-BC; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) has been developed as a commercially available chromogenic medium for the detection of *B. cereus*. Since this medium can detect *B. cereus* as specific blue-green colored colonies without interference with other colonies grown, this media can easily differentiate typical *B. cereus* with no skills and experiences.

In these days, Japanese standard methods for food hygiene control have been shifted to internationally validated ISO standard method to keep international cooperativeness. Even though there are several kinds of commercially available selective media in Japan,
the ISO 7932: 2004 recommends to use only MYP as selective agar medium (International Organization for Standardization, 2006). Hence, these commercially available non-MYP selective media need to be verified their compatibility with ISO 7932: 2004. The aim of this study was to compare the performance of 3 kinds of selective media in the detection and enumeration of B. cereus using ISO 7932: 2004 method.

For the inclusivity study, 3 kinds of media were assessed using 25 B. cereus strains. The exclusivity of these media was assessed using 50 strains (25 gram-positive and 25 gram-negative bacteria). In both inclusivity and exclusivity study, each strain tested was cultured twice in tryptic soy broth (Becton Dickinson, Sparks, MD, USA) at 35°C for 18 h and was then subjected to 10-fold serial dilution in buffered peptone water (BPW; Merck KGaA, Darmstadt, Germany). One hundred microliters of each suspension were inoculated onto each of the three plates of MYP, NGKG, and X-BC, and spread using sterilized glass rod to confirm repeatability of each medium. After MYP, NGKG, X-BC, and TSA were incubated at 30 ± 2°C for 24 ± 2h, 32 ± 2°C for 24 ± 2h, 35 ± 2°C for 24 ± 2h, 32 ± 2°C for 24 ± 2h, and 35 ± 2°C for 24 ± 2h, respectively, the colonies on each medium were observed and counted. The numbers obtained from each medium were converted into log CFU/ml, and the mean log CFU/ml and standard deviation (SD) were then calculated for each strain tested. A one-way analysis of variance (ANOVA) was performed to determine differences among 3 selective media compared using Microsoft Excel 2013 at the significance level of P = 0.05.

In the inclusivity study, all of 25 B. cereus strains grew and formed pink colored colonies with egg yolk reaction on both MYP and NGKG as shown in Figure 1. And all B. cereus strains tested grew and formed blue-green colored colonies on X-BC (Figure 1). As shown in Table 2, the numbers (mean log CFU/ml ± SD) of B. cereus obtained from MYP, NGKG and X-BC were 7.24 ± 0.32, 7.26 ± 0.32, and 7.22 ± 0.31, respectively. These numbers were equivalent with that obtained from non-selective TSA (7.26 ± 0.31). The ranges of recovered B. cereus numbers (mean log CFU/ml) from MYP, NGKG and X-BC were 6.81 to 7.77, 6.67 to 7.71, and 6.66 to 7.73, respectively. There is no statistically significant difference (p > 0.05) in recovered B. cereus numbers among MYP, NGKG and X-BC.

The results of exclusivity study are shown in Table 3. Of the 50 strains tested, 11, 7, 3 strains were allowed to grow on MYP, NGKG and X-BC. A total of B. thuringiensis tested grew and formed typical B. cereus colonies on all 3 media. All the other grown bacteria formed atypical B. cereus colonies and were easily differentiated from typical B. cereus colonies. The numbers (mean log CFU/ml ± SD) of all non-B. cereus bacteria grew on MYP, NGKG and X-BC were equivalent with that obtained from non-selective TSA.

Since the ISO 7932: 2004 recommends to use only MYP for the detection of B. cereus, NGKG and X-BC needed to be compared with MYP using artificially contaminated food matrix according to ISO 7932: 2004. In this method comparison study, fried rice, plain rice, fried noodle and potato salad, in which there is no B. cereus, were used as artificially contaminated

| Ingredient | Formulation (g/L) |
|------------|------------------|
| Peptone    | MYP 10.0 NGKG 1.0 X-BC 10.0 |
| Meat peptone | MYP 1.0 NGKG 15.0 X-BC 10.0 |
| Meat extract | MYP 1.0 NGKG 0.5 X-BC 1.0 |
| Yeast extract | MYP 0.5 NGKG 0.5 X-BC 0.5 |
| Sodium chloride | MYP 10.0 NGKG 4.0 X-BC 5.0 |
| D-mannitol | MYP 10.0 NGKG 0.0 X-BC 0.0 |
| Glycine | MYP 3.0 NGKG 0.0 X-BC 3.0 |
| Polymyxin B | MYP 100k units NGKG 50k units X-BC 100k units |
| Phenol red | MYP 0.025 NGKG 0.025 X-BC 0.025 |
| Egg yolk (100% conversion) | MYP 25mL NGKG 20mL X-BC 20mL |
| Selective agent | MYP 0.01 NGKG 0.01 X-BC 0.01 |
| Chromogenic substrate | MYP 0.15 NGKG 0.15 X-BC 0.15 |
| Agar | MYP 15.0 NGKG 18.0 X-BC 15.0 |

pH: MYP 7.2 ± 0.2, NGKG 6.8 ± 0.2, X-BC 7.0 ± 0.2

Formulation of each selective medium was referred from manufacturer information.

**FIG. 1.** The typical appearance of B. cereus on various selective media. B. cereus ATCC 11778 formed pink colored colonies with egg yolk reaction on MYP (A) and NGKG (B), and blue-green colored colonies on X-BC (C) after 24h of incubation at 30, 32, 35°C, respectively.
### COMPARISON OF SELECTIVE MEDIA FOR B. CEREUS

#### TABLE 2. Growth of Bacillus cereus on various selective media

| Strains of Bacillus cereus | MYP          | NGKG         | X-BC         | TSA          |
|---------------------------|--------------|--------------|--------------|--------------|
| B. cereus ATCC 10876      | 7.10 ± 0.01  | 7.14 ± 0.02  | 7.10 ± 0.10  | 7.07 ± 0.04  |
| B. cereus ATCC 11778      | 6.95 ± 0.08  | 6.95 ± 0.04  | 6.80 ± 0.16  | 6.94 ± 0.06  |
| B. cereus ATCC 13061      | 6.81 ± 0.06  | 6.67 ± 0.09  | 6.66 ± 0.10  | 6.75 ± 0.04  |
| B. cereus ATCC 14579      | 7.63 ± 0.02  | 7.63 ± 0.07  | 7.55 ± 0.06  | 7.63 ± 0.05  |
| B. cereus ATCC 33019      | 7.43 ± 0.23  | 7.46 ± 0.21  | 7.55 ± 0.20  | 7.34 ± 0.04  |
| B. cereus NFH A-102       | 7.02 ± 0.06  | 7.08 ± 0.03  | 6.97 ± 0.02  | 7.08 ± 0.04  |
| B. cereus NFH A-104       | 7.06 ± 0.13  | 7.09 ± 0.10  | 6.91 ± 0.05  | 7.17 ± 0.09  |
| B. cereus NFH A-105       | 7.23 ± 0.03  | 7.32 ± 0.08  | 7.25 ± 0.10  | 7.31 ± 0.09  |
| B. cereus NFH A-107       | 7.34 ± 0.08  | 7.40 ± 0.11  | 7.33 ± 0.04  | 7.52 ± 0.15  |
| B. cereus NFH A-113       | 6.99 ± 0.01  | 6.96 ± 0.03  | 7.00 ± 0.05  | 6.99 ± 0.03  |
| B. cereus NFH A-114       | 6.89 ± 0.13  | 6.89 ± 0.10  | 6.90 ± 0.07  | 6.78 ± 0.10  |
| B. cereus NFH A-116       | 7.11 ± 0.05  | 7.16 ± 0.06  | 7.11 ± 0.06  | 7.19 ± 0.10  |
| B. cereus NFH A-118       | 7.66 ± 0.04  | 7.71 ± 0.02  | 7.53 ± 0.07  | 7.64 ± 0.05  |
| B. cereus NFH A-119       | 7.13 ± 0.08  | 7.23 ± 0.07  | 7.24 ± 0.12  | 7.22 ± 0.05  |
| B. cereus NFH A-121       | 7.18 ± 0.04  | 7.31 ± 0.04  | 7.25 ± 0.03  | 7.30 ± 0.09  |
| B. cereus NFH A-123       | 7.24 ± 0.01  | 7.22 ± 0.05  | 7.23 ± 0.06  | 7.21 ± 0.04  |
| B. cereus NFH A-125       | 6.92 ± 0.07  | 7.00 ± 0.12  | 6.84 ± 0.04  | 7.00 ± 0.13  |
| B. cereus NFH A-126       | 7.77 ± 0.04  | 7.71 ± 0.03  | 7.73 ± 0.09  | 7.72 ± 0.04  |
| B. cereus NFH A-128       | 7.22 ± 0.04  | 7.36 ± 0.08  | 7.33 ± 0.02  | 7.27 ± 0.06  |
| B. cereus NFH B-101       | 7.14 ± 0.04  | 7.11 ± 0.06  | 7.23 ± 0.08  | 7.16 ± 0.10  |
| B. cereus NFH B-102       | 6.95 ± 0.14  | 7.05 ± 0.18  | 7.02 ± 0.14  | 7.11 ± 0.10  |
| B. cereus NFH B-106       | 7.04 ± 0.02  | 7.04 ± 0.06  | 7.05 ± 0.04  | 7.11 ± 0.10  |
| B. cereus NFH B-108       | 7.13 ± 0.12  | 7.13 ± 0.05  | 7.10 ± 0.10  | 7.06 ± 0.08  |
| B. cereus NFH B-118       | 7.72 ± 0.10  | 7.71 ± 0.05  | 7.61 ± 0.01  | 7.64 ± 0.04  |
| B. cereus NFH B-119       | 7.13 ± 0.08  | 7.23 ± 0.07  | 7.24 ± 0.12  | 7.22 ± 0.05  |
| Overall                   | 7.24 ± 0.32  | 7.26 ± 0.32  | 7.22 ± 0.31  | 7.26 ± 0.31  |

#### p value (ANOVA)

- vs. MYP: 0.64
- vs. NGKG: 0.64
- vs. X-BC: 0.68

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food matrix, respectively, since these food matrices have high frequency for B. cereus food poisoning (Bennett et al., 2015; Shinagawa, 1990). For method comparison study, spores of three strains of B. cereus (ATCC13061, ATCC 10876, NFH A-128) were used for making artificially contaminated food matrix. Spore solution was made according to following procedure. After B. cereus was cultured at 30°C for 10 days, spore of B. cereus was collected into sterilized distilled water. Subsequently, collected spore was washed 3 times.
TABLE 3. Exclusivity of non-\(B. \text{cereus}\) bacteria tested on various selective media a

| Strains tested b | MYP | Mean log CFU/ml ± SD (colony color) c | NGKG | X-BC | TSA |
|------------------|-----|-------------------------------------|------|------|-----|
| Gram-positive bacteria |     |                                     |      |      |     |
| \(Aerococcus viridans\) ATCC 10400 | ND c | ND | ND | ND | 9.43 ± 0.02 |
| \(Bacillus circulans\) ATCC 4516 | 7.75 ± 0.10 (w) | ND | ND | ND | 7.90 ± 0.06 |
| \(B. \text{licheniformis}\) ATCC 12759 | 8.06 ± 0.09 (w) | ND | ND | ND | 8.03 ± 0.03 |
| \(B. \text{megaterium}\) ATCC 9885 | ND | ND | ND | ND | 7.30 ± 0.03 |
| \(B. \text{pumilus}\) ATCC 14884 | 7.91 ± 0.13 (w) | 7.80 ± 0.11 (w) | ND | 7.98 ± 0.08 |
| \(B. \text{subtilis}\) ATCC 11774 | 7.54 ± 0.01 (w) | ND | ND | ND | 7.56 ± 0.08 |
| Strains tested |     |                                     |      |      |     |
| \(H. \text{teramura et al.}\) |     |                                     |      |      |     |
| \(C. muytjensii\) ATCC 51329 | ND | ND | ND | 9.05 ± 0.05 |
| \(C. sakazakii\) | ND | ND | ND | 9.00 ± 0.10 |
| \(Klebsiella \text{aerogenes}\) JCM 15521 | ND | ND | ND | 8.93 ± 0.06 |
| \(L. \text{casei}\) ATCC 334 | ND | ND | ND | 9.42 ± 0.02 |
| \(L. \text{fermentum}\) IFO 14513 | 8.55 ± 0.06 (cl) | w (cl) | ND | 8.56 ± 0.10 |
| \(L. \text{plantarum}\) ATCC 8014 | w (cl) | ND | ND | ND | 8.17 ± 0.02 |
| \(L. \text{sakei}\) ATCC 15521 | ND | ND | ND | 9.03 ± 0.02 |
| \(L. \text{lactis}\) subsp. \(lactis\) NBRC 12007 | w (cl) | ND | ND | ND | 9.58 ± 0.06 |
| \(L. \text{thermophilus}\) ATCC 19258 | ND | ND | ND | 9.70 ± 0.06 |
| \(S. \text{epidermidis}\) ATCC 12228 | ND | 8.43 ± 0.03 (w) | ND | 8.36 ± 0.04 |
| \(S. \text{hominis}\) subsp. \(hominis\) JCM 2419 | 8.85 ± 0.06 (cr) | w (cr) | ND | 9.10 ± 0.10 |
| \(Streptococcus\) warneri JCM 2415 | 8.80 ± 0.12 (w) | ND | ND | 8.82 ± 0.10 |
| Gram-negative bacteria |     |                                     |      |      |     |
| \(Aeromonas salmonicida\) ATCC 7965 | ND | 9.20 ± 0.03 (w) | ND | 9.25 ± 0.05 |
| \(Citrobacter \text{koseri}\) JCM 1658 | ND | ND | ND | 8.08 ± 0.06 |
| \(C. \text{freundii}\) ATCC 8090 | ND | ND | ND | 9.41 ± 0.05 |
| \(Cronobacter \text{dublinensis}\) subsp. \(dublinensis\) JCM 16467 | ND | ND | ND | 8.87 ± 0.08 |
| \(C. \text{mutans}\) ATCC 51329 | ND | ND | ND | 9.05 ± 0.05 |
| \(C. \text{sakazakii}\) ATCC 29544 | ND | ND | ND | 9.00 ± 0.10 |
| \(Klebsiella \text{aerogenes}\) JCM 1235 | ND | ND | ND | 9.20 ± 0.10 |
| \(Enterobacter\) cloacae subsp. \text{cloacae}\) IFO 13535 | 9.08 ± 0.02 (w) | 9.03 ± 0.06 (w) | ND | 9.25 ± 0.04 |
| \(Escherichia \text{coli}\) ATCC25922 | ND | ND | ND | 8.09 ± 0.05 |
| \(Klebsiella \text{oxytoca}\) JCM1665 | ND | ND | ND | 9.25 ± 0.06 |
| \(K. \text{pneumoniae}\) subsp. \(pneumoniae\) ATCC 35657 | ND | ND | ND | 9.18 ± 0.03 |
| \(Klebsiella \text{ascorbata}\) JCM 1681 | ND | ND | ND | 9.05 ± 0.07 |
| \(Leclercia \text{adcarboxylate}\) JCM 1667 | ND | ND | ND | 8.98 ± 0.06 |
| \(Morganella \text{morganii}\) subsp. \text{morganii}\) NBRC 3168 | ND | ND | ND | 9.19 ± 0.10 |
| \(Pantoea \text{agglomerans}\) JCM 1236 | ND | ND | ND | 9.08 ± 0.04 |
| \(Pluralibacter\) gergoviae ATCC 33028 | ND | ND | ND | 9.01 ± 0.06 |
| \(Proteus \text{hauseri}\) ATCC 13315 | ND | ND | ND | 8.69 ± 0.12 |
| \(Providencia\) rettgeri ATCC 9250 | ND | ND | ND | 8.99 ± 0.18 |
| \(Pseudomonas\) aeruginosa ATCC 10145 | ND | ND | ND | 8.69 ± 0.12 |
| \(Raoultella\) ornitholytica NBRC 105727 | ND | ND | ND | 8.16 ± 0.04 |
| \(R. \text{planticola}\) NBRC 14939 | ND | ND | ND | 9.22 ± 0.04 |
| \(Salmonella\) enterica serovar Enteritidis IFO 3313 | ND | ND | ND | 8.81 ± 0.03 |
| \(S. \text{enterica}\) serovar Typhimurium ATCC 7823 | ND | ND | ND | 8.79 ± 0.02 |
| \(Serratia\) marcescens ATCC 13880 | ND | ND | ND | 8.94 ± 0.06 |
| \(Yersinia\) enterocolitica subsp. \text{enterocolitica}\) ATCC 9610 | ND | ND | ND | 8.10 ± 0.09 |

aMYP, NGKG, X-BC, and TSA was cultured at 30 ± 2°C for 24 ± 2h, 32 ± 2°C for 24 ± 2h, 35 ± 2°C for 24 ± 2h, and 35 ± 2°C for 24 ± 2h, respectively.
bStrains were derived from ATCC (American Type Culture Collection), IAM (Institute of Molecular and Cellular Biosciences, The University of Tokyo), IFO (Institute for Fermentation Osaka, Japan), JCM (Japan Collection of Microorganisms) and NBRC (NITE Biological Resource Center, Japan). cND: not detected.
dw: poor and very small.
eCharacteristics indicate colony appearance: w, white; pEY, pink and egg yolk reaction; bg, blue-green; cl, colorless; cr, cream.
using sterilized distilled water, and was then heated at 70°C for 40 min. The spore of each B. cereus strain was spiked into each 25 g of sample at the following levels, respectively: high (7 log CFU/g), medium (5 log CFU/g) and low (3 log CFU/g) per each food matrix, according to AOAC validation guideline and ISO 16140-2 (AOAC International, 2012; International Organization for Standardization, 2016). Sample preparation and inoculation were according to ISO 7932: 2004. In brief, after spiked food samples were preserved at 5°C for 3 days, they were homogenized with 9-fold volume of BPW at 60 sec. using homogenizer (MASTICATOR 400S, IUL, S. A., Barcelona, Spain). Each homogenized sample was subjected to 10-fold serial dilution in BPW, 100 μl of each dilution was then inoculated onto 2 plates of MYP, NGKG, and X-BC and spread using sterilized glass rod, respectively. After each medium was incubated at same condition with inclusivity/exclusivity study, respectively, typical B. cereus colonies grown were counted. Results obtained from each medium tested were converted into log CFU/g of B. cereus per each food matrix tested. The mean log CFU/g and standard deviation (SD) at each contamination level of food matrix tested were then calculated. Data analysis was conducted under the assumption that MYP, NGKG, and X-BC were reference method, candidate method 1, candidate method 2, respectively according to the validation of AOAC International Performance Tested Methods (Teramura et al., 2018). In addition, ANOVA was performed to determine differences of candidate methods against reference method using Microsoft Excel 2013 at the significance level of P = 0.05. The method comparison study was summarized in Table 4. The ranges of mean log CFU/g ± SD of MYP, NGKG, and X-BC were 3.53 to 7.73, 3.53 to 7.71, and 3.48 to 7.57, respectively. And ranges of SD of those were 0.05 to 0.25, 0.09 to 0.29, and 0.05 to 0.26, respectively. Overall mean log CFU/g ± SD of MYP, NGKG, and X-BC were 5.57 ± 1.63, 5.56 ± 1.62, and 5.52 ± 1.59, respectively. These results showed that MYP, NGKG, and X-BC had equivalent range of mean log CFU/g ± SD regardless of food matrix. These results also suggested that these 3 selective media had similar accuracy and repeatability. Further, range of mean of differences between MYP and NGKG, and MYP and X-BC were -0.45 to 0.07 and -0.44 to 0.04 and were within ± 0.5 log. In a microbiological viewpoint, a difference of ≤ 0.5 log is not considered to be practically significant (Teramura et al., 2018). Hence, NGKG and X-BC had no differences with MYP as a reference in point of accuracy.

Figure 2 showed plots of recovered B. cereus numbers (Log CFU/g) between each candidate method and reference method for all food matrix. The slopes and

| Food matrix | Inoculation level | N | MYP (Reference) | NGKG (Candidate 1) | Mean diff. (Can. 1 - Ref.) | X-BC (Candidate 2) | Mean diff. (Can. 2 - Ref.) |
|-------------|------------------|---|----------------|-------------------|--------------------------|----------------|--------------------------|
|             |                  |   | Mean log CFU/g ± SD | Mean log CFU/g ± SD |                          | Mean log CFU/g ± SD |                          |
| Fried rice  | High             | 3 | 7.73 ± 0.25       | 7.71 ± 0.22       | -0.02                    | 7.55 ± 0.25       | -0.18                    |
|             | Med              | 3 | 5.70 ± 0.20       | 5.65 ± 0.24       | -0.05                    | 5.65 ± 0.26       | -0.05                    |
|             | Low              | 3 | 3.82 ± 0.05       | 3.81 ± 0.09       | -0.01                    | 3.84 ± 0.09       | 0.02                     |
| Plain rice  | High             | 3 | 7.72 ± 0.13       | 7.27 ± 0.16       | -0.45                    | 7.28 ± 0.16       | -0.44                    |
|             | Med              | 3 | 5.51 ± 0.08       | 5.48 ± 0.11       | -0.03                    | 5.42 ± 0.13       | -0.09                    |
|             | Low              | 3 | 3.55 ± 0.23       | 3.62 ± 0.14       | 0.07                     | 3.59 ± 0.14       | 0.04                     |
| Fried noodle| High             | 3 | 7.64 ± 0.10       | 7.69 ± 0.15       | 0.05                     | 7.57 ± 0.05       | -0.07                    |
|             | Med              | 3 | 5.37 ± 0.25       | 5.36 ± 0.22       | -0.01                    | 5.35 ± 0.24       | -0.02                    |
|             | Low              | 3 | 3.65 ± 0.24       | 3.63 ± 0.23       | -0.02                    | 3.63 ± 0.24       | -0.02                    |
| Potato salad| High             | 3 | 7.47 ± 0.25       | 7.41 ± 0.29       | -0.06                    | 7.39 ± 0.26       | -0.08                    |
|             | Med              | 3 | 5.59 ± 0.15       | 5.59 ± 0.09       | 0.00                     | 5.49 ± 0.09       | -0.10                    |
|             | Low              | 3 | 3.53 ± 0.16       | 3.53 ± 0.14       | 0.00                     | 3.48 ± 0.22       | -0.05                    |
| Overall     |                  | 36| 5.57 ± 1.63       | 5.56 ± 1.62       | -0.01                    | 5.52 ± 1.59       | -0.05                    |

*Method comparison study was conducted using spiked food matrix with spores of B. cereus ATCC 13061, ATCC 10876 and NFH A-128, respectively. MYP, NGKG, and X-BC was cultured at 30 ± 2°C for 24 ± 2h, 32 ± 2°C for 24 ± 2h, and 35 ± 2°C for 24 ± 2h, respectively. Spiked bacterial levels were at high (7 log CFU/g), medium (5 log CFU/g) and low (3 log CFU/g), respectively.
intercepts of linear regression line between NGKG and MYP, and X-BC and MYP were 1.01 and -0.02, and 1.02 and -0.06 as shown in Figure 2. Further, these slopes and intercepts were close to 1.00 and 0.00, respectively. The both correlation coefficient (r) between NGKG and MYP, and X-BC and MYP were 0.999. ANOVA showed that both NGKG and X-BC had no statistically significant differences (p > 0.05) with MYP.

In this study, our findings demonstrated that 3 kinds of commercially available selective media for the enumeration of \textit{B. cereus} had equivalent performance in count. However, even though NGKG had similar detection principle, NGKG showed more selective than MYP in exclusivity study. For X-BC, since it had most selective among 3 selective media and can differentiate \textit{B. cereus} from other competitive bacteria due to specific color development, it was easiest to read results in method comparison study. Moreover, it suggested that MYP and NGKG have a possibility of miscount for typical colonies, since typical egg yolk reaction of both MYP and NGKG showed tendency to spread and interfere with other grown colonies. On the other hand, X-BC did not show widespread of typical colored colonies in addition to its excellent selectivity. Hence, it seemed that X-BC has a less possibility of miscount even if there are large number of \textit{B. cereus} grown on the medium.

However, X-BC allowed \textit{B. thuringiensis} to form typical \textit{B. cereus} colonies as well as other 2 selective media in spite of containing specific chromogenic substrate for \textit{B. cereus}, since it is known well that \textit{B. thuringiensis} has same phenotypic biochemical characteristics (Vilas-Bôas et al., 2007). ISO 7932: 2004 is established as a method for enumeration of presumptive \textit{B. cereus}. Hence, it needs confirmation test to distinguish \textit{B. cereus} from closely related Bacillus strain such as \textit{B. thuringiensis}. Even though \textit{B. thuringiensis} is hard to be distinguished on microbiological media due to its phenotypic biochemical characteristics, \textit{B. thuringiensis} can be differentiated by the observation of parasporal insecticidal crystal toxin using microscopy or the amplification of specific \textit{cry} gene which is coded crystal toxin by PCR base techniques (Vilas-Bôas et al., 2007).

Our results showed the numbers of \textit{B. cereus} obtained from NGKG and X-BC had no differences with that from MYP even though incubation temperature for each medium was different. Therefore, it suggested that NGKG and X-BC had a possibility to be alternative media in ISO 7932: 2004 method.

In conclusion, we compared the performances of MYP, NGKG, and X-BC which are commercially available in Japan and demonstrated that NGKG and X-BC are equivalent to or better performance than MYP which is recommend in ISO 7932: 2004 when using the standard ISO 7932: 2004 method in fried rice, plain rice, fried noodle, and potato salad. Therefore, it is suggested that NGKG and X-BC can be adapted to the ISO 7932: 2004 method for the enumeration of \textit{B. cereus} in selected food matrix as well as MYP.

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