INTRODUCTION

Refrigerated pasteurized processed foods have recently gained popularity due to their high sensorial, textural, and nutritional attributes (Membre et al., 2006). In addition, these foods are not typically processed with high-heat sterilization, as in retort treatment. The pasteurization of these products is commonly performed at 90°C for 10 min (Membre et al., 2006). However, a large variety of spore formers have often been detected in various refrigerated pasteurized processed foods such as vegetable purees, ready-to-eat meals, fish surimi, and milk, especially when they are close to the recommended “sell-by” date (Carlin et al., 2000; Choma et al., 2000; Coton et al., 2011; Guinebretiere et al., 2001; Hamasaki et al., 2006; Ranieri et al., 2012; Kobayashi et al., 2016; Helmond et al., 2017). This suggests that conventional pasteurization for such refrigerated processed food is not enough to completely inactivate the bacterial spores. The vegetative growth of the surviving spore formers causes quality losses such as souring, package swelling, off-odor formation, and increased turbidity (Guinebretiere et al., 2001; Hamasaki et al., 2006; Kobayashi et al., 2016; Meer et al., 1991). Thus, it is important to inactivate spore and/or suppress spore growth for preventing food spoilage during refrigerated storage.

Bacillus cereus is a spore-forming bacterium detected in pasteurized processed foods stored at refrigeration temperatures. It is recognized that their spores survive after conventional pasteurizing treatments (Silva and Gibbs, 2010). Other Bacillus spp. and Paenibacillus spp. have also been detected as dominant species in various pasteurized processed foods stored at low temperatures (Carlin et al., 2000; Coton et al., 2011;
Materials and Methods

Bacterial strains

In total, 72 strains of *Bacillus* spp. and 45 strains of *Paenibacillus* spp. were used in this study (Tables 1 and 2). All strains were confirmed to have a growth ability at 10°C. Of these, 36 *Bacillus* strains and 12 *Paenibacillus* strains were obtained from the NITE Biological Resource Center (Ibaraki, Japan), the Japan Collection of Microorganisms (Ibaraki, Japan), and the Genebank Project (Ibaraki, Japan). The other 69 strains had been previously isolated from food processing environments (cookware, manufacturing equipment, storage tanks, and conveyor belts) and chilled foods, and identified by comparing the approximately 500 bp sequences of 16S rDNA with the nucleotide sequence registered in GenBank (https://www.ncbi.nlm.nih.gov/).

Preparation of spore suspensions

All bacterial strains were grown in tryptic soy broth (BD, New Jersey, USA) supplemented with 6 g/L yeast extract (BD) at 30°C for 24–48 h. Fresh cultures of the strains were spread onto nutrient agar medium containing nutrient broth (NB; Eiken Chemical, Tokyo, Japan) and 15 g/L agar (FUJIFILM Wako Pure Chemical, Osaka, Japan) and incubated at 30°C for 2–3 weeks. After confirmation of spore formation with a differential interference microscope, spores were scraped off with a spreader and suspended in cold sterile distilled water (SDW). The obtained spores were washed with cold SDW (1500 x g, 10 min, 10°C, 5 times) according to the method described by Yamazaki et al. (1997). The final pellets were resuspended in 30 mL cold SDW and stored at 4°C until use. Spore suspensions were heated at 75°C for 15 min prior to the subsequent experiments.

Determination of spore heat resistance

Spore heat resistance was determined using a method described by Yamazaki et al. (1997), with few modifications. A 500 μL aliquot of phosphate buffer (1/15M, pH 7.0) containing approximately 6 log spores/mL was centrally injected into a sterile glass tube (7 mm i.d., 9 mm o.d., 120 mm length, IWATA Glass Industrial, Osaka, Japan). The tubes were sealed by pulling the end off in a gas oxygen flame and were then heated to 85.0–97.5°C with a stirred program heater (TR-4AR, AS ONE, Osaka, Japan) in a bath (SB-24, TOKYO RIKAKIKAI, Tokyo, Japan) filled with polyethylene glycol (KO10002000, King Manufacturing Company, Osaka, Japan). After heat treatment, the tubes were immediately cooled in ice water, and the tops were cut off using an ampoule cutter (B type, Maruemu, Osaka, Japan). A 100 μL spore suspension was serially diluted with 900 μL phosphate-buffered saline, plated on Standard Methods Agar (Nissui Pharmaceutical, Tokyo, Japan) and incubated at 30°C for 1–3 days. The experiments were performed three times for each strain. The Dt-value was defined as minutes of heat treatment at temperature t for surviving microbes to drop by 1 log10 cycle. The z-value was defined as the temperature for the Dt-value to change by 1 log10 cycle.

Determination of growth ability at refrigeration temperatures

Heated spore suspensions (10 μL) were inoculated into 990 μL of NB (pH 7.1) dispensed in 48-well microplates (AGC Techno Glass, Shizakura, Japan), at an initial inoculum size of 5 log spores/mL, and the plates were incubated at 4, 6, 8, and 10°C, respectively. Microbial growth was determined by the medium turbidity observed visually after 4–28 days of incubation. Experiments were performed in three wells for each strain.

Results and Discussion

The spore D value of *Bacillus* spp. and *Paenibacillus* spp.

Tables 1 and 2 show the spore D values of *Bacillus* and *Paenibacillus* strains at various temperatures. The spore heat resistance of *B. cereus* has been commonly evaluated at 90°C (Daelman et al., 2013; Choma et al., 2000; Dufrenne et al., 1995; Luu-Thi et al., 2014; Meer et al., 1991; Samapundo et al., 2011). The spore D10°C of 34 *Bacillus* and 23 *Paenibacillus* strains tested in this
### TABLE 1. Spore heat resistance and growth ability at refrigeration temperatures of Bacillus strains.

| Tested strain | D value (min) | z value (°C) | Growth days at refrigeration temperatures<sup>a</sup> |
|---------------|---------------|--------------|-----------------------------------------------|
|               | 87.5°C | 90.0°C | 92.5°C | 95.0°C | 97.5°C | 4°C | 6°C | 8°C | 10°C |
| **B. cereus group** |          |          |          |          |        |     |     |     |     |
| *B. cereus* NBRC3001 |          |          |          |          |        |     |     |     |     |
| *B. cereus* NBRC3002 | 14.2±0.1 | 6.8±0.9 | 2.9±0.3 | 7.2 |          | 14 | 10 | 4 | 4 |
| *B. cereus* NBRC3003 |          |          |          |          |        |     |     |     |     |
| *B. cereus* NBRC3004 |          |          |          |          |        |     |     |     |     |
| *B. cereus* NBRC3005<sup>T</sup> |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118482 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118502 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118512 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118515 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118519 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118521 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118523 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118524 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118525 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118526 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118527 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118532 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118590 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118591 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118593 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118594 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118596 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118597 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118598 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118601 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF118607 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF301697 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF301699 |          |          |          |          |        |     |     |     |     |
| *B. cereus* MAFF520024 |          |          |          |          |        |     |     |     |     |
| *B. thuringiensis* NBRC101235<sup>T</sup> | 15.2±0.6 | 6.3±0.3 | 3.0±0.1 | 7.1 |          | 10 | 4 | 4 | 4 |
| *B. weihenstephanensis* NBRC101238<sup>T</sup> |          |          |          |          |        |     |     |     |     |
| *B. cereus sensu latio* No.5 | 10.6±0.5 | 4.8±0.2 | 2.3±0.2 | 7.6 |          | 11 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.6 | 11.1±0.0 | 4.9±0.3 | 2.3±0.3 | 7.3 |          | 11 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.9 | 17.4±1.2 | 6.7±0.9 | 3.4±0.3 | 7.0 |          | 11 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.271 | 14.0±1.7 | 7.8±1.3 | 3.2±0.3 | 7.8 | 21 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.275 | 44.0±8.5 | 20.9±6.3 | 5.8±1.1 | 5.7 |          | 7 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.301 | 10.7±2.3 | 4.9±1.5 | 2.1±0.2 | 7.1 |          | 7 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.503 | 20.1±3.8 | 7.9±1.7 | 3.3±0.8 | 6.3 |          | 4 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.591 | 19.3±2.2 | 8.3±1.5 | 3.3±0.2 | 6.6 |          | 11 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.1071 | 16.8±1.2 | 6.2±0.4 | 3.7±0.4 | 7.6 |          | 7 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.1072 |          |          |          |          |        | 7 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.10711 |          |          |          |          |        | 7 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.10810 |          |          |          |          |        | 7 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.1093 |          |          |          |          |        | 4 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.1099 |          |          |          |          |        | 7 | 4 | 4 | 4 |
| *B. cereus sensu latio* No.2611 |          |          |          |          |        | 14 | 7 | 4 | 4 |
| **B. megaterium** |          |          |          |          |        |     |     |     |     |
| *B. megaterium* NBRC15308<sup>T</sup> | 14.2±0.1 | 6.8±0.9 | 2.9±0.3 | 7.2 |          | 14 | 10 | 7 | 7 |
| *B. megaterium* No.3 |          |          |          |          |        | 14 | 7 | 4 | 4 |
| *B. megaterium* No.5 |          |          |          |          |        | 11 | 4 | 4 | 4 |
| *B. megaterium* No.17 | 7.7±0.3 | 3.7±0.4 | 1.8±0.0 | 7.9 |          | 21 | 4 | 4 | 4 |


The days observed visible medium turbidity with microbial growth.

No turbidity observed in 28 days of incubation.

Means: standard deviation.

The blank spaces of D value and z value were not measured or calculated.

The spore heat resistance and growth ability at refrigeration temperatures of some strains was not determined in this study, their spore D 90°C of of some strains was not determined in this study, their spore heat resistance at 90°C, calculated from corresponding z values, were compared. The spore D 90°C of 23 Bacillus strains (67.6%) was less than 15 min. In particular, the heat resistance of many strains including approximately half of the strains of the B. cereus group and all strains of B. megaterium, B. pumilus, and B. simplex, were distributed in this range. Eight B. cereus strains were found to form higher heat-resistant spores (D 90°C = 25 min or greater) than the other Bacillus strains. Surprisingly, spores of two B. cereus strains (MAFF118590 and MAFF118598) showed high heat resistance (82.9 and 91.6 min, respectively). The spore D 90°C of 17 Paenibacillus strains (73.9%) was less than 15 min. Moreover, heat resistance of the spores of P. terrae (JCM11466) and strain No. 9) and Paenibacillus sp. (JCM13343 and strain No. 1) was equivalent to that of spores of the aforementioned eight B. cereus strains. Among the spores assessed, spores of Paenibacillus sp. JCM13343 exhibited the highest heat resistance (D 90°C = 136.1 min).

The distribution of the spore D 90°C of the B. cereus group in our study was similar to the results of previous studies. In many previous studies, the spore D 90°C of B. cereus, which could grow at refrigeration temperatures, was found to be less 15 min (Choma et al., 2000; Dufrénne et al., 1995; Luu-Thi et al., 2014; Meer et al., 1991). Samapundo et al. (2014) reported that B. cereus spores were reduced 1.5 log in liquid media (pH 7, Aw 0.990) after heating at 90°C for 10 min, suggesting a D 90°C of approximately 6.7 min. Additionally, a few strains with a spore D 90°C of approximately 90 min or more than 100 min have been reported (Daelman et al., 2013; Dufrénne et al., 1995).

There are few reports on the spore heat resistance of Bacillus spp. and Paenibacillus spp., except for B. cereus, which can grow at refrigeration temperatures. Meer et al. (1991) reported that the D 90°C of B. pumilus spores isolated from milk was 5.1 min. Helmond et al. (2017) and Inatsu et al. (2017) reported that the D 90°C of Paenibacillus spores isolated from spoiled processed foods was 3.9–5.0 min. Our results showed that the spore D 90°C of Bacillus and Paenibacillus strains that grow at refrigeration temperatures were less than 15 min. However, P. terrae and two strains of Paenibacillus sp. were found to form higher heat-resistant spores (D 90°C = 27 min or greater) than the other Paenibacillus strains.
TABLE 2. Spore heat resistance and growth ability at refrigeration temperatures of *Paenibacillus* strains.

| Tested strain | D value (min) | z value | Growth days at refrigeration temperatures<sup>a</sup> |
|---------------|---------------|---------|--------------------------------------------------|
|               | 85.0°C | 87.5°C | 90.0°C | 92.5°C | 95.0°C | 97.5°C | 4°C | 6°C | 8°C | 10°C |
| *P. odorifer* JCM13339 | 9.3±0.2<sup>d</sup> | 4.1±0.4 | 2.3±0.1 | | | | | | | |
| *P. odorifer* JCM21743<sup>T</sup> | 9.2±0.1 | 4.4±0.2 | 2.2±0.1 | | | | | | | |
| *P. odorifer* No.3 | 7.7±0.8 | 3.8±0.4 | 2.0±0.1 | | | | | | | |
| *P. polymyxa* JCM2507<sup>T</sup> | 6.3±0.2 | 3.6±0.2 | 1.8±0.0 | | | | | | | |
| *P. polymyxa* JCM20106 | 6.7±0.4 | 3.4±0.2 | 1.6±0.1 | | | | | | | |
| *P. polymyxa* JCM20385 | 13.9±0.1 | 6.9±0.7 | 3.0±0.1 | | | | | | | |
| *P. polymyxa* No.1 | 12.5±0.7 | 6.0±0.4 | 2.9±0.2 | | | | | | | |
| *P. polymyxa* No.7 | 13.7±0.5 | 6.7±0.7 | 3.1±0.2 | | | | | | | |
| *P. polymyxa* No.11 | 25.1±2.3 | 12.2±0.6 | 6.3±0.5 | | | | | | | |
| *P. polymyxa* No.952 | 11.6±0.3 | 6.1±0.2 | 2.6±0.1 | | | | | | | |
| *P. polymyxa* No.1095 | 10.9±0.4 | 6.3±0.1 | 2.6±0.2 | | | | | | | |
| *P. terrae* JCM11466<sup>T</sup> | 27.5<sup>d</sup> | 11.7±0.3 | 5.6±0.3 | 2.3±0.2 | 7.0 | | | | | |
| *P. terrae* No.2 | | | | | | | | | | |
| *P. terrae* No.8 | | | | | | | | | | |
| *P. terrae* No.9 | | | | | | | | | | |
| Others | | | | | | | | | | |
| *P. caespitis* No.112 | | | | | | | | | | |
| *P. chibensis* JCM9905<sup>T</sup> | | | | | | | | | | |
| *P. humicus* No.58 | 48.9±9.2 | 20.3±2.7 | 9.8±0.9 | | | | | | | |
| *P. humicus* No.65 | | | | | | | | | | |
| *P. pabuli* NBRC13638<sup>T</sup> | | | | | | | | | | |
| *P. pabuli* No.411 | 21.7±1.5 | 9.9±0.6 | 4.1±0.4 | | | | | | | |
| *P. taichungensis* No.5 | | | | | | | | | | |
| *P. taichungensis* No.39 | 11.6±0.5 | 6.1±0.3 | 2.6±0.2 | | | | | | | |
| *P. taichungensis* No.295 | | | | | | | | | | |
| *P. taichungensis* No.303 | 19.9±0.7 | 9.0±0.7 | 4.4±0.6 | | | | | | | |
| *P. xylanexedens* No.1 | | | | | | | | | | |
| *P. xylanexedens* No.4 | | | | | | | | | | |
| *P. xylanexedens* No.274 | | | | | | | | | | |
| *P. xylanexedens* No.284 | | | | | | | | | | |
| *Paenibacillus* sp. JCM13338 | 3.1±0.4 | | | | | | | | | |
| *Paenibacillus* sp. JCM13341 | 24.3±3.2 | 10.3±1.5 | 5.2±0.6 | | | | | | | |
| *Paenibacillus* sp. JCM13342 | 38.5±3.3 | 15.7±2.1 | 7.5±0.3 | | | | | | | |
| *Paenibacillus* sp. JCM13343 | 136.1 | 54.8±12.8 | 21.4±1.3 | 8.5±1.6 | 6.2 | | | | | |
| *Paenibacillus* sp. No.1 | 48.3 | 19.5±1.3 | 8.8±0.4 | 3.3±0.0 | 6.5 | | | | | |
| *Paenibacillus* sp. No.2 | | | | | | | | | | |
| *Paenibacillus* sp. No.4 | 11.7±1.3 | | | | | | | | | |
| *Paenibacillus* sp. No.6 | | | | | | | | | | |
| *Paenibacillus* sp. No.7 | 9.3±0.5 | 4.5±0.0 | 2.0±0.1 | | | | | | | |
| *Paenibacillus* sp. No.9 | | | | | | | | | | |
| *Paenibacillus* sp. No.111 | 5.5±0.4 | | | | | | | | | |
| *Paenibacillus* sp. No.122 | | | | | | | | | | |
| *Paenibacillus* sp. No.173 | | | | | | | | | | |
| *Paenibacillus* sp. No.174 | | | | | | | | | | |
| *Paenibacillus* sp. No.281 | | | | | | | | | | |
| *Paenibacillus* sp. No.293 | 5.4±0.4 | | | | | | | | | |

<sup>a</sup>The days observed visible medium turbidity with microbial growth.
<sup>b</sup>Mean±standard deviation.
<sup>c</sup>No turbidity observed in 28 days of incubation.
<sup>d</sup>D<sub>90</sub>C written in italics were calculated from z value.

The blank spaces of D value and z value were not measured or calculated.
Thus, it is presumed that the spore heat resistance of \textit{Bacillus} and \textit{Paenibacillus} strains that could grow at 10°C are similar to each other.

The spore z value of \textit{Bacillus} spp. and \textit{Paenibacillus} spp.

Tables 1 and 2 show the spore z values of the \textit{Bacillus} and \textit{Paenibacillus} strains. Among the 22 strains of the \textit{B. cereus} group, the spore z values were as follows: 5.7°C, strain No. 275; 6–7°C, 3 strains, 7–8°C, 15 strains; and 8–9°C, 3 strains. The four \textit{B. megaterium} strains, three \textit{B. pumilus} strains and three \textit{B. simplex} strains showed spore z values in the range of 7–8°C. Among the 22 \textit{Paenibacillus} strains, the spore z values of three strains were in the range of 6–7°C, of 13 strains in the range of 7–8°C, of 5 strains in the range of 8–9°C, and that of \textit{P. polymyxa} JCM2507 was 9.1°C. Most strains of either genera had spore z values in the range of 7–8°C, and there were no strains in which the spore z value exceeded 10°C.

The spore z values of \textit{B. cereus} group, \textit{B. pumilus}, \textit{B. simplex}, and \textit{B. subtilis}, which can grow at refrigeration temperatures, have been reported to be in the range of 7–10°C (Baril et al., 2012; Condon-Abanto et al., 2016; Coton et al., 2011; Daelman et al., 2013; Meer et al., 1991; Samapundo et al., 2011; Trunet et al., 2015). Additionally, the spore z values calculated from the reported D values (Helmond et al., 2017) of four \textit{Paenibacillus} isolates from refrigerated ready-to-eat meals were 5.7–9.4°C. Our results are similar to those of previous studies. Thus, the spore z value of \textit{Bacillus} spp. and \textit{Paenibacillus} spp., which can grow at refrigeration temperatures, may be in the range of 7–10°C, irrespective of genus or species.

Growth ability of \textit{Bacillus} spp. and \textit{Paenibacillus} spp. at refrigeration temperatures

Most strains of \textit{Bacillus} (64 strains out of 72) and \textit{Paenibacillus} (41 strains out of 45) grew within 7 days at 10°C (Tables 1 and 2). Thus, it was considered that the growth ability at 10°C for both genera was not different. The lowest temperature at which each strain could grow among the tested temperatures is summarized in Figure 2, which indicates that most of the \textit{Paenibacillus} strains (32 strains out of 45) and only a few strains of the \textit{B. cereus} group (3 strains out of 48) could grow at 4°C. While some \textit{Paenibacillus} strains, including all strains of \textit{P. odorifer} (3 strains out of 3), grew within 14
days at 4°C, three strains of the *B. cereus* group grew only after 21 days or later.

Buehler et al. (2018) reported that *P. odorifer* had a high growth ability at lower temperatures, and the lag time of *P. odorifer* was very short compared to that of *B. weihenstephanensis*. Additionally, all *B. simplex* strains (6 strains out of 6) assessed in our study grew at lower temperatures (Fig. 2). *Bacillus simplex* is widely distributed in nature (Mani et al., 2016; Schwartz et al., 2013; Sikorski and Nevo, 2005). The five isolates, except for JCM12307, were obtained from pasteurized milk, processed foods, and agricultural food processing environments. In contrast, only a few reports have indicated that *B. simplex* is a causative spoilage microorganism in pasteurized processed foods (Coton et al., 2011; Helmond et al., 2017). Based on these findings, *Paenibacillus* was presumed to grow better at lower temperatures than *Bacillus*, although their growth abilities were not significantly different at 10°C.

**Relationship between spore heat resistance and growth ability at refrigeration temperatures**

The relationship between spore D<sub>90°C</sub> and the lowest temperature at which each strain could grow is summarized in Figure 3. Among the 34 *Bacillus* strains, spores of 8 *B. cereus* strains showed higher heat resistance than others but did not grow at temperatures below 10°C. All 12 *Bacillus* strains that grew at 6°C or lower were found to have a spore D<sub>90°C</sub> of less than 15 min. Conversely, four strains of *Paenibacillus* that formed high heat-resistant spores similar to the eight *B. cereus* strains could grow at 6°C or lower. In particular, the spores of *Paenibacillus* sp. JCM13343 showed the highest heat resistance among all the tested strains and grew at 4°C. In another study, *Paenibacillus* spp. were isolated as the predominant species from pasteurized ready-to-eat meals and milk stored at 7°C or lower (Helmond et al., 2017; Ranieri et al., 2012). Based on these findings, *Paenibacillus* was presumed to grow better at lower temperatures than *Bacillus*, although their growth abilities were not significantly different at 10°C.
six Bacillus strains, when $D_{90°C}$ values of Bacillus and Paenibacillus strains, showed no growth at temperature below 6°C in this study. The growth ability at refrigeration temperature of B. cereus strains that formed high heat-resistant spores was determined in previous studies. Dufrenne et al. (1995) reported that B. cereus strain forming spores with high heat resistance ($D_{90°C} > 100$ min) hardly germinated in skim milk at 7°C. In addition, Daelman et al. (2013) showed that heat-treated B. cereus spores did not grow at 10°C in liquid media with modified pH and Aw mimicking the conditions of pasteurized refrigerated processed foods. These results suggest that the high heat-resistant spores of B. cereus strains do not germinate or grow in processed foods during refrigerated storage, even if the bacteria are able to grow at refrigeration temperatures. Conversely, Paenibacillus sp. JCM13343 and P. terrae strain No. 9, which formed high heat-resistant spores, spoiled the processed food during refrigerated storage (Hamasaki et al., 2006; Kobayashi et al., 2016). Therefore, Paenibacillus spores with higher heat resistance survive conventional pasteurization and show vegetative growth during refrigerated storage because they can grow at lower temperatures (e.g., 6°C or lower).

In this study, we revealed that many Paenibacillus strains had a psychrotrophic character, and spores of some of these strains showed high heat resistance similar to the heat-resistant spores of B. cereus that did not grow at temperatures below 10°C. Therefore, Paenibacillus strains with higher heat resistant-spor e are thought to withstand inadequate pasteurization processes and germinate and grow in refrigerated processed food, leading to food spoilage. Therefore, Paenibacillus should be considered as one of the target species for microbiological control in refrigerated processed food as well as B. cereus. This study may provide useful information for developing a proper pasteurization procedure to eliminate spore-forming bacteria that can grow at refrigeration temperatures.

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