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Biochemical property identification of 10 strains of *Bacillus thuringiensis* and 10 strains of *Bacillus cereus* (7 strains of non-emetic and 3 strains of emetic type) by API test

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Abstract The objective of this study was to identify the fermentation characteristics of *Bacillus thuringiensis* and emetic, non-emetic *Bacillus cereus* using analytical profile index (API) test. Ten strains of *B. thuringiensis* and 10 strains of *B. cereus* including 3 strains of emetic type were used at the same concentrations. The differences of fermentation characteristics between the *B. thuringiensis* and *B. cereus* was not obvious, but the differences between the non-emetic and emetic *B. cereus* were distinctive. Seven among 50 substrates were negative for all non-emetic *B. cereus* strains and positive for all emetic strains, and three substrates among additional 12 substrates had the same tendency. From these differences, 3 emetic *B. cereus* strains were not indicated as *B. cereus* by API test. These results indicate that API test is not a suitable method to identify some strains of emetic *B. cereus*, and the distinctive differences in substrate utilization can be used to improve selective media.

Keywords: *Bacillus cereus*, *Bacillus thuringiensis*, API test, emetic, biochemical property

Introduction

Food poisoning has occurred worldwide. In particular, the food poisoning issue has been hot issue in Korea every year, especially in the summer seasons. Food poisoning is defined as any infectious or toxic disease suspected to have been caused by a toxin produced by microbes or microorganisms after eating food (Korean Food Sanitation Act, 2020). Food poisoning can be divided into physical, chemical, and microbiological outbreaks depending on the type of harmful materials (Ryu and Lee, 2011). Even though some chemicals include animal, vegetable, and artificial compounds cause food poisoning, most food poisonings are caused by microorganisms (Lee et al., 1996). In Korea, 58.6% of 221 food poisoning cases between 1981 and 1990 were caused by bacteria. Likewise, 61.3% of foodborne illnesses were caused by bacteria in Japan (Lee et al., 1996). More specifically, microbiological hazards are divided into bacterial and viral hazards, and bacterial hazards can be subdivided into toxic and infectious types (Bean and Griffin, 1990).

Typical examples of food poisoning bacteria include *Escherichia coli* O157:H7, *Salmonella*, *Listeria*, and *Bacillus cereus* (Kobayashi et al., 2009). Among these bacterial pathogens, *Bacillus* has a unique characteristic of aerobic, endospore-forming bacteria (Goto et al., 2000). Most *Bacillus* spp. is naturally widespread due to spore formation and various external factors (Iurlina et al., 2006), which include *B. subtilis*, *B. cereus, B. mycoides, B. anthracis, B. thuringiensis, B. pseudomycoides,* and *B. weihenstephanensis* (Callahan et al., 2009). Among the various species, *B. subtilis* and *B. cereus* groups are related to food deterioration and food poisoning, respectively. These *Bacillus* spp. have unique characteristic of spore-forming ability resulting high resistance which makes it difficult to control (Kim et al., 2009).

Foodborne illness by *B. cereus* can be divided into diarrheal and emetic type. The diarrheal syndrome is caused by ingestion of food contaminated by pathogenic microorganisms that produce toxins in the body (Granum and Lund, 1997). This syndrome is caused by enterotoxins, which are produced in the small intestine by pathogenic bacteria. The diarrheal syndrome mainly occurs by meat products, soups, vegetables, and pudding whereas the emetic syndrome occurs by fried rice, rice, pasta, pastry, and noodles (Granum and Lund, 1997). The enterotoxin produced by diarrheal type *B. cereus* promotes the cAMP system, causing diarrhea (Laohachai et al., 2003). The infectious dose is known to as 5-7 log CFU/g, and the incubation period is known to as 8-16 hours (Granum and Lund, 1997). On the other hand, the emetic syndrome is caused by the ingestion of food containing toxins produced by pathogenic microorganisms (Ehling-Schulz et al., 2004). The mechanism is caused by the intake of heat-resistant toxins produced by bacteria. These heat-resistant toxins are stable at pH 2 to pH 11, and not decomposed by digestive enzymes (Webb et al., 2019). The toxins stimulate the vagus nerve and is transmitted to the vomiting heart of the brain, causing severe vomiting (Popoff and Poulain, 2010). Toxin production is most active at 12-22°C (From et al., 2005). The required concentration of bacteria is known to more than 5-8 log CFU/g in food products, which is produced by growing for 0.5 to 5 hours (Granum and Lund, 1997).

B. thuringiensis, belonging to the *B. cereus* group, is an insect pathogenic microorganism (Ahn and Lee, 2010). The crystal

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protein of *B. thuringiensis* reacts with the mid-intestinal fluid of insects and is decomposed into toxic proteins, forming a hole in the cell membrane, causing sepsis and exhibiting insecticidal activity (Chen et al., 2007). The identification of *Bacillus* spp. is traditionally confirmed through a number of analyses based on the morphological and biochemical properties. For example, Mannitol-egg yolk-Polymyxin B (MYP) medium has been widely used which represents a precipitation zone surrounding the estimated colony due to the hydrolysis of egg yolk, and the estimated colony cannot produce acid from mannitol (Hendriksen and Hansen, 2011). However, it has been indicated that *B. thuringiensis* and *B. cereus* grow in the same form in the MYP medium (Hendriksen and Hansen, 2011). It is well known that microorganisms in *B. cereus* groups are genetically very similar with lecithinase-positive, Voges Prausker-positive, and mannitol negative properties (Jeon and Park, 2010). Another method of detecting the *B. cereus* group to which *B. cereus* and *B. thuringiensis* belong is to use a PCR method that amplifies the specific and unique DNA sequence of the target bacteria. The most commonly used gene segment is the 16S rRNA. However, 16S rRNA has limited ability to differentiate *B. cereus* group bacteria (Chang et al., 2003). Moreover, it has been reported that several strains of *B. thuringiensis* contain genes known to be related to the pathogenesis of *B. cereus* (Jeon and Park, 2010). Biochemical tests such as API 50 CHB and API 20E are known to be used for identification of *B. cereus* group (Carpana et al., 1995). In API test, strips consists of wells containing dehydrated substrates which has specific relation with fermentation of carbohydrate or catabolism of amino acid/proteins. However, to the best of out knowledge research related to the fermentation characteristics of *B. thuringiensis* and emetic, non-emetic *B. cereus* are limited. In the present study, fermentation characteristics of 10 strains of *B. thuringiensis* and 10 strains of *B. cereus* (7 strains of non-emetic and 3 strains of emetic type) were identified and analyzed.

Materials and Methods

Bacterial strain

Seven strains of non-emetic *B. cereus*; ATCC 14579, ATCC 10876, ATCC 13061, ATCC 21768, ATCC 10987, ATCC 3674, ATCC 1094, three strain of emetic *B. cereus*; A, B, C (isolated from specimens of patients who ate grain products were obtained from Dankook University (Cheonan, South Korea). Ten strains of *B. thuringiensis*; KCTC 3452, KCTC 1508, KCTC 1510, KCTC 1511, KCTC 1512, KCTC 1513, KCTC 1514, KCTC 1515, KCTC 1517, KCTC 1524 were obtained from Korean Collection for Type Cultures (KCTC, Joenbuk, Korea). PCR analysis was used to verify the *B. cereus* strains (data not shown).

Bacterial cultures and cell suspension

B. cereus and *B. thuringiensis* were incubated on Tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks Glencoe, MD). A single colony cultivated from TSA was inoculated into 5 mL of Tryptic soy broth (TSB; Difco, Franklin Lakes, New Jersey, USA) and incubated at 30°C for 24 h. After incubation, the bacterial cultures were mixed with a vortexer and centrifuged for 20 min at 5000×*g*. The pellets were resuspended using 9 mL of 0.2% sterile peptone water (PW; Difco).

API kit preparation

API 50CHB, 20E kit and suspension medium, McFarland 2, 20E kit reagent (James, NIT 1, 2, VP 1, 2, TDA), mineral oil were purchased from BioMerieux (Marcy-L'Etoile, France). The kits except mineral oil were stored at 5°C until use. The turbidity of McFarland 2 was measured immediately after purchase and used as a reference to adjust cell concentration.

Cell concentration adjustment

Bacteria frozen at −24°C was dissolved in glycerol stock at room temperature, then taken one loopful from stock and inoculated into 5 mL of TSB broth. After incubation at 30°C for 24 h, the turbidity was adjusted following the manufacturer's guide using turbidmeter (TU-2016, Lutron Electronic, Taiwan) and the reference (McFarland 2). The turbidities in the API suspension medium were adjusted to be 35.00-45.00 considering the turbidity of McFarland 2 (40.64). In this wasy, API 20E kit suspension medium and API 50CHB kit suspension medium were adjusted to be the same concentration of the bacteria

Inoculation and incubation

The API 50CHB kit was inoculated with 100 µL of the pre-treated bacterial solution in 0 (control) to 49 tubes. After inoculating, the kits were incubated at 30°C for 24 h. After incubation, the results of the 50CHB kit was identified by putting + or − regarding the table on the API website. In the case of the 20E kit, the results were read 15 min after adding the additional 20E kit reagent according to the manufacturer's instructions. The CIT, CP, and GEL tubes of the 20E kit were fully inoculated according to the manufacturer's instructions, while ADH, LDC, ODC, H_2S, and URE tubes were filled with mini oil to create anaerobic conditions. The remaining tubes were inoculated with 100 µL inoculum each. As additional 20E kit reagents, TDA reagents were added to TDA tube, James reagents were added to IND tube, VP 1 and VP 2 reagents were added to VP tube, and finally, NIT 1 and NIT 2 reagents were added to GLU tube. After adding the 20E kit reagent, the results were analyzed using the API reading table presented on the API site, and the sequence of + and − for each strain was entered. After entering the positive or negative results, strains were analyzed and verified through API program. The analysis results were derived in % ID format.

Results and Discussion

Fermentation properties of non-emetic/emetic *B. cereus* and *B. thuringiensis* using API 50CHB

Fermentation properties of non-emetic/emetic *B. cereus* and *B. thuringiensis* were identified by API 50CHB (Table 1). Our results indicated that variations in non-emetic *B. cereus* were observed for Arbutin (24), Esculin ferric citrate (25), Salicin (26), Amidon (starch, 36), and Glycogen (37) whereas the remaining substrates

Table 1. Fermentation ability of non-emetic *B. cereus*, emetic *B. cereus*, and *B. thuringiensis* identified by API 50CHB

API 50CHB	Control	Glycerol	Erythritol	D-arabinose	L-arabinose	D-ribose	D-xylose	L-xylose	D-adonitol	Methyl-β-D-xylopyranoside
Non-emetic *B. cereus*	0/7[1]	0/7	0/7	0/7	0/7	7/7	1/7	0/7	0/7	0/7
Emetic *B. cereus*	0/3	3/3	0/3	2/3	3/3	3/3	3/3	0/3	0/3	0/3
Total *B. cereus*[2]	0/10	3/10	0/10	2/10	3/10	10/10	4/10	0/10	0/10	0/10
B. thuringiensis	3/10	0/10	0/10	0/10	0/10	8/10	0/10	0/10	0/10	0/10

API 50CHB	D-galactose	D-glucose	D-fructose	D-mannose	L-sorbose	L-rhamnose	Dulcitol	Inositol	D-mannitol	D-sorbitol
Non-emetic *B. cereus*	0/7	7/7	7/7	1/7	0/7	0/7	0/7	0/7	0/7	0/7
Emetic *B. cereus*	3/3	3/3	3/3	3/3	0/3	3/3	3/3	0/3	3/3	3/3
Total *B. cereus*	3/10	10/10	10/10	4/10	0/10	3/10	3/10	0/10	3/10	3/10
B. thuringiensis	0/10	9/10	9/10	4/10	0/10	0/10	0/10	0/10	0/10	0/10

API 50CHB	methyl-α-D-manno-pyranoside	methyl-α-D-glucopy-ranoside	N-acetyl-gluco-samine	Amygdalin	Arbutin	Esculin ferric citrate	Salicin	D-cellobiose	D-maltose	D-lactsoe (bovine origin)
Non-emetic *B. cereus*	0/7	0/7	7/7	0/7	3/7	4/7	3/7	2/7	7/7	0/7
Emetic *B. cereus*	0/3	0/3	3/3	0/3	2/3	3/3	3/3	0/3	3/3	3/3
Total *B. cereus*	0/10	0/10	10/10	0/10	5/10	7/10	6/10	2/10	10/10	3/10
B. thuringiensis	0/10	0/10	10/10	1/10	6/10	10/10	8/10	1/10	10/10	0/10

API 50CHB	D-melibiose	D-saccharose (sucrose)	D-trehalose	Inulin	D-melezitose	D-raffinose	Amidon (starch)	Glycogen	Xylitol	Gentiobiose
Non-emetic *B. cereus*	0/7	6/7	6/7	0/7	0/7	0/7	3/7	3/7	0/7	0/7
Emetic B. cereus	3/3	2/3	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Total *B. cereus*	3/10	8/10	9/10	0/10	0/10	0/10	3/10	3/10	0/10	0/10
B. thuringiensis	0/10	9/10	10/10	0/10	0/10	0/10	9/10	9/10	0/10	0/10

API 50CHB	D-turanose	D-lyxose	D-tagatose	D-fucose	L-fucose	D-arabitol	L-arabitol	potassium glucoNaTe	potassium 2-keto-gluconate	potassium 5-keto-gluconate
Non-emetic *B. cereus*	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7
Emetic *B. cereus*	0/3	0/3	0/3	0/3	3/3	0/3	0/3	1/3	0/3	0/3
Total *B. cereus*	0/10	0/10	0/10	0/10	3/10	0/10	0/10	1/10	0/10	0/10
B. thuringiensis	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

[1)]Positive results/total results
[2)]Total *B. cereus* means the sum of non-emetic and emetic *B. cereus* results.

did not show any differences depending on the strains. Previously, Logan and Berkeley (1984) also reported that fermentation abilities were different for Galactose, Inositol, α-Methyl-D-glucoside, and Amygdalin when 119 *B. cereus* (non-emetic) were tested. In the case of the emetic *B. cereus,* our results showed that fermentation properties were different for D-arabinose (3), Arbutin (24), D-saccharose (sucrose, 31), and Potassium gluconate (47) depending on the strains whereas those of remaining substrates were consistent regardless of strains. In Logan and Berkeley (1984)'s study, fermentation properties of Arbutin, Cellobiose, D-raffinose, Glycogen, D-turanose, L-fucose and Gluconate were different depending on the strains. These results indicate that the fermentation property would be different depending on the number and type of strains.

In the present study, distinctive differences were observed for the fermentation properties between the emetic *B. cereus* and non-emetic *B. cereus* (Table 1). Fermentation property was different for substrates such as Glycerol, L-arabinose, D-xylose, D-galactose, D-mannose, L-rhamnose, Dulcitol, D-mannitol, D-sorbitol, D-lactose (bovine origin), D-mellobiose, and L-fucose. Emetic *B. cereus* ferments (+) these substrates whereas non-emetic *B. cereus* cannot ferment (–) the substrates. In the case of D-xylose and D-mannose, six out of seven strains of non-emetic *B. cereus* showed negative results whereas one strain presented a positive result. A previous study by Logan and Berkeley (1984) indicates that the fermentation properties of D-mannose, Inositol, α-Methyl-D-glucoside, Amygdalin, Salicin, Lactose, Melezitose, Starch, and L-fucose were different between the emetic and non-emetic strains. In addition, Kim et al (2004a)'s study separated *B. cereus* from food and identified the characteristics of the pathogens using API 50CHB. When API 50CHB results of Kim et al (2004a)'s study was compared with the results by total *B. cereus* from the present study, it showed a perfectly match for Erythritol (–), D-ribose (+), L-xylose (–), Adonitol (–), β-Methyl-D-xyloside (–), D-glucose

(+), D-fructose (+), L-sorbose (–), Inositol (–), α-Methyl-D-mannoside (–), α-Methyl-D-glucoside (–), N-Acetyl glucosamine (+), Maltose (+), Melezitose (–), Raffinose (–), Xylitol (–), Gentiobiose (–), D-turanose (–), D-lyxose (–), D-tagatose (–), D-fucose (–), D-arabitol (–), L-arabitol (–), 2-Keto gluconate (–), and 5-Keto gluconate (–). On the other hand, results for Amygdalin and Inulin are totally inconsistent between the two studies. The present study showed negative (–) for Amygdalin and Inulin whereas positive (+) results were observed in Kim et al. (2004b) study. Our research was conducted with seven non-emetic *B. cereus* and three emetic *B. cereus*. On the other hand, *B. cereus* was separated from the raw beef of jangzorim in Kim et al. (2004b) study. Since the result of Kim et al. (2004b)'s study is based on only one strain of *B. cereus*, it is expected that the difference in strains would affect the differences.

The API test of *Bacillus* spp. has been reported previously. For example, Jeon and Park (2010) conducted a toxin gene analysis of *B. cereus* and *B. thuringiensis* separated from cooked rice. API 50CHB was conducted for nine strains of *B. cereus*, and the results of six of the 50 CHB substrate were recorded. In the Jeon and Park (2010)'s study, fermentation properties (positive/total) were as follows: Salicin (8/9), Amidon (1/9), Glycogen (1/9), D-turanose (6/9), D-tagatose (2/9), L-fucose (1/9) while Salicin (6/10), Amidon (3/10), Glycogen (3/10), D-turanose (0/10), D-tagatose (0/10), and L-fucose (3/10). Wu et al. (2014) identified enterotoxin production and antimicrobial susceptibility of *B. cereus* BY06 isolated from pigs with diarrheal disease**.** In the Wu et al. (2014)'s study, the isolated *B. cereus* BY06 and *B. cereus* BY12 were subjected to a biochemical test through API 50CHB. When API 50CHB results were compared with the total *B. cereus* results with our study, results for following substrates matched perfectly: Erythritol (–), D-ribose (+), L-xylose (–), D-adonitol (–), Methyl-ß-D-xylopyranoside (–), D-glucose (+), D-fructose (+), L-sorbose (–), Methyl-α-D-mannopyranoside (–), Methyl-α-D-glucopyranoside (–), D-maltose (+), Inulin (–), D-melezitose (–), Xylitol (–), Gentibiose (–), D-turanose (–), D-lyxose (–), D-tagatose (–), D-fucose (–), D-arabitol (–), L-arabitol (–), Potassium 2-kegto-gluconate (–), and Potassium 5-ketogluconate (–). There was no substrate that has total inconsistency between the two studies. These results indicate that the results by API test would be different to where the pathogen was isolated.

In the present study, some substrates showed different fermentation properties between *B. thuringiensis* and total *B. cereus.* However, the properties were almost identical for *B. thuringiensis* and *B. cereus*. These similar characteristics made it difficult to distinguish the two pathogens not only by using commercial MYP (Hendriksen and Hansen, 2011) but also by PCR (Chang et al., 2003). In the Ahn and Lee (2010)'s study, API 50CHB was performed to separate *B. thuringiensis* BT17 strain having excellent insecticidal properties from the carcasses of silkworm moth larvae. When the results of the present study were compared with the Ahn and Lee (2010)'s study, 33 of the 49 substrates contained in API 50CHB showed the same results whereas Glycerol, L-xylose, Adonitol, D-mannitol, α-Methyl-D-Mannoside, α-Methyl-D-glucoside, and Esculin showed different aspects. The reaction of the aforementioned substrates was '–' in the present study whereas it was '+' in the Ahn and Lee (2010)'s study. On the other hand, Esculin '+' in the present study and '–' in (Ahn and Lee, 2010) study. These results indicate that results can be varied which strains of microorganisms were used in the experiment. In this regard, more comparative and systematic studies are needed for the further study.

Fermentation properties of non-emetic/emetic *B. cereus* and *B. thuringiensis* using API 20E

Fermentation properties of non-emetic *B. cereus* and *B. thuringiensis* were identified by API 20E (Table 2). Distinctive differences were observed for the fermentation properties between the emetic *B. cereus* and non-emetic *B. cereus*. Substrates such as 2-Nitrophenyl β-D-galactopyranoside, L-lysine, Trisodium citrate, and L-tryptophane (9) showed different fermentation aspects between the emetic and non-emetic strains, which indicates that these substrates can be used to distinguish non-emetic *B. cereus* from emetic *B. cereus*. Three emetic *B. cereus* ferment (+) these substrates while seven non-emetic *B. cereus* cannot ferment (–) the substrates. Previously, Logan and Berkeley (1984) reported that fermentation properties of ADH (L-arginine) and URE (urease) were different between the emetic and non-emetic strains when 119 *B. cereus* (non-emetic) and 30 emetic *B. cereus* were tested. These differences are may be due to the different strains of *B. cereus* used in the experiments.

In the present study, variations were observed for fermentation ability of L-arginine and D-glucose among non-emetic *B. cereus*, but the remaining substrates did not show any differences depending on the strains. Similarly, Logan and Berkeley (1984)

Table 2. Fermentation ability of non-emetic *B. cereus*, emetic *B. cereus*, and *B. thuringiensis* identified by API 20E

API 20E	2-nitro-phenyl-β-D-galactopyranoside	L-arginine	L-lysine	L-ornithine	Trisodium citrate	Sodium thiosulfate	Urea	L-trypto-phane	L-trypto-phane	Sodium pyruvate	Gelatin (bovine origin)	D-glucose
Non-emetic *B. cereus*	0/7[1]	5/7	0/7	0/7	0/7	0/7	0/7	7/7	0/7	7/7	7/7	4/7
Emetic *B. cereus*	3/3	1/3	3/3	0/3	3/3	0/3	0/3	3/3	3/3	3/3	3/3	3/3
Total *B. cereus*[2]	3/10	6/10	3/10	0/10	3/10	0/10	0/10	10/10	3/10	10/10	10/10	7/10
B. thuringiensis	0/10	8/10	0/10	0/10	1/10	0/10	0/10	10/10	0/10	10/10	10/10	6/10

[1)]Positive results/total results
[2)]Total *B. cereus* means the sum of non-emetic and emetic *B. cereus* results.

Table 3. Identification percentage (% ID) of *B. cereus* by API test

B. cereus strain	Non-emetic (ATCC)							Emetic		
	10876[c]	13061[c,d,e]	14579[c]	21768[c,d,e]	10987[c,d]	3674[c,d]	1094[c]	A	B	C
B. cereus[a]	14.5	80.6	51.7	83.0	96.6	77.3	85.5	ND[b]	ND[b]	ND[b]
B. mycoides	85.1	-	47.1	-	-	-	14.3			
B. firmus	-	17.5	-	7.0	3.3	22.5	-			
B. anthracis	-	1.8	-	9.8	-	-	-			

[a)]Sum of *B. cereus* 1 and 2
[b)]ND: Not determined
[c)]Possibility of *Bacillus thuringiensis*
[d)]Possibility of *Bacillus megaterium*
[e)]Highly pathogenic organism

Table 4. Identification percentage (% ID) of *B. thuringiensis* by API test

B. thuringiensis strain (KCTC)	1508[b]	1510[b]	1511[b]	1512[b]	1513[b]	1514[b]	1515[b,c,d]	1517[b]	1524[b]	3452[b]
B. cereus[a]	51.1	14.5	15.1	93.1	80.8	15.7	45.5	38.8	40.5	18.1
B. mycoides	48.7	85.1	84.7	6.6	-	84.0	-	60.4	59.0	81.0
B. firmus	-	-	-	-	-	-	35.5	-	-	-
B. anthracis	-	-	-	-	-	-	17.9	-	-	-
Brevibacillus laterosporus	-	-	-	-	16.9	-	-	-	-	-

[a)]Sum of *B. cereus* 1 and 2
[b)]Possibility of *Bacillus thuringiensis*
[c)]Possibility of *Bacillus megaterium*
[d)]Highly pathogenic organism

reported that fermentation abilities were different for ADH (L-arginine), and URE (urease) when 119 *B. cereus* (non-emetic) were used. In the case of the emetic *B. cereus*, our results showed that fermentation properties were different for L-arginine whereas remaining substrates were consistent regardless of strains. In Logan and Berkeley (1984)'s study, fermentation properties of 2-Nitrophenyl β-D-galactopyranoside, L-lysine, Trisodium citrate, and L-tryptophane (9) were different depending on the strains. From the results of API 50CHB and 20E tests, the distinctive differences between the non-emetic and emetic *B. cereus* were indicated which can be used for the development of selective media.

Identification percentage (% ID) using API test

Identification percentages were identified by enter the results of API 50CHB and API 20E (Tables 3 and 4). The seven non-emetic *B. cereus* strains commonly identified the results of "Possibility of *Bacillus thuringiensis*" in their notes (Table 3). Through this, we concluded that that there is a limit to distinguish *B. cereus* from *B. thuringiensis* by API test. All 7 strains of non-emetic *B. cereus* were identified by the IDs of *B. cereus*, *B. mycoides, B. firmus,* and *B. anthracis*, which could be confirmed to be very similar to the results of *B. thuringiensis* in Table 4. In the Aruwa and Olatope (2015)'s study, API test was conducted using 5 *B. cereus*, and all 5 IDs were confirmed as *B. cereus*, which was different from our results. It was also derived with two or three IDs per strain. In addition, when *B. thuringiensis* or *B. cereus* results are identified, there are cases where the ID is differently identified with *B. cereus* 1 and *B. cereus* 2. In the present study, these results were repsented as one result and expressed as *B. cereus* ID by summing up each value. However, the three strains of emetic *B. cereus* have not been determined in the API program in the present study, which was indicated as "not determineded (ND)" section in Table 3. In Kim et al. (2004a)'s study, *B. cereus* contamination status and growth pattern analysis in ready-to-eat foods were conducted by isolating *B. cereus* from 240 food samples. *B. cereus* KCCM 40935, *B. cereus* KCTC 1661, *B. cereus* KCTC 1092, and *B. cereus* KCTC 3062, which were used as standard strains, were confirmed using API as a result, "*B. cereus, B. mycoides*". In addition, *B. cereus* isolated from seafood, packaged meals, and RTE foods were represented as "*B. cereus, B. mycoides, B. anthracis*". Each API ID was derived from one to two. Non-emetic *B. cereus* used in our research was "*B. cereus, B. mycoides, B, anthracis*, and *B. firmus*" and was similar to the Kim et al. (2004a)'s study. In the Kim et al. (2004a)'s study and our study, the % ID value was slightly different for each strain, which is thought to be due to the difference in the *B. cereus* strain used in each experiment. Meanwhile, Kang et al., (2008) reported the usefulness of PCR analysis for the identification of *B. cereus* group. The strains used in the experiment (*B. cereus* KCTC 3624, *B. cereus* BGSC 6E1, *B. cereus* KCTC 1014, *B. cereus* KCTC 1094, and *B. cereus* KCTC 3062) was sub-cultured on nutrient agar medium and API 50CHB. The result by API 50CHB and API 20E was "*B. cereus* 1, *B. anthracis*, *B. mycoides*". The results were confirmed within the range similar to our study, and the % ID value was slightly different. In other words, although it is known that approximate *Bacillus* species can be identified through API 50CHB and API 20E, the limit of identification is found through API, especially for *B. cereus*, which is difficult to identify.

Table 4 represents the identification percentage (% ID) value of 10 strains *B. thuringiensis*. Ten strains of *B. thuringiensis* were

commonly derived as "Possibility of *B. thuringiensis*". All 10 strains were identified in the categories of *B. cereus, B. mycoides, B. firmus, B. anthracis,* and *Brevibacillus laterosporus*. Most *B. thuringiensis* strains were often identified with *B. cereus* and *B. mycoides*, derived by two or three IDs per strain. Previously, Aruwa and Olatope (2015) conducted experiments using four *B. thuringiensis* separated from food which is determined within the categories of *B. mycoides, B. cereus, B. anthracis,* and *B. pseudomycoides*. These results are in agreement with our results except for *B. pseudomycoides*. As a result of API test, it was confirmed that *B. thuringiensis* was not derived from the identity strain and that only "Possibility of *Bacillus thuringiensis*" was derived from the reference. In other words, API tests alone has a limitation to accurately identify *B. thuringiensis*. In addition, *B. thuringiensis* has similar characteristic with other *B. cereus* group such as *B. mycoides* and *B. anthracis* (Slamti and Lereclus, 2002), so we were able to identify the results of *B. cereus* group bacteria in our results. Previously, Kang et al. (2008) conducted experiment with *B. thuringiensis* strains (*B. thuringiensis* BGSC 4AB1, *B. thuringiensis* BGSC 4AJ1, *B. thuringiensis* BGSC 4AQ1, *B. thuringiensis* BGSC 4AS1, *B. thuringiensis* BGSC 4CC1, and *B. thuringiensis* DNS 2046) and confirmed these strains as "*B. cereus* 1, *B. mycoides*" using API 50CHB and API 20E. In our study, it was derived from the range of "*B. cereus, B. mycoides, B. firmus, B. anthracis,* and *Brevibacillus laterosporus*" which is thought to be different in range because of the different strains of *B. thuringiensis* used. These results indicate that API test has limitation to identify *B. thuringiensis* strains directly and further confirmation experiment is needed.

Conclusion

API 50CHB and API 20E test were conducted to identify the fermentation characteristics of 7 non-emetic *B. cereus* strains, 3 strains of emetic *B. cereus*, and 10 *B. thuringiensis* strains in the present study. API test resulted in similar fermentation characteristics of *B. cereus* and *B. thuringiensis* whereas there was a distinctive difference between the emetic *B. cereus* and non-emetic *B. cereus*. The results from API test indicated the 7 non-emetic *B. cereus* as *B. cereus*, but 3 emetic *B. cereus* strains were not identified as *B. cereus* from API test. Therefore, API test should be modified in order to identify the emetic *B. cereus,* or other confirmation methods are needed to ensure microbiological safety from some emetic *B. cereus* strains. Finally, this study is expected to contribute to the development of a selective/differential medium that can distinguish emetic *B. cereus* and non-emetic *B. cereus*.

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Conflict of interest

The authors declare no conflict of interest.

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