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The Phenotypic and Genotypic Characterization of Korean Isolates of *Cronobacter* spp. (*Enterobacter sakazakii*)

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This study was conducted to investigate the phenotypic and genotypic characteristics of Korean isolates of *Cronobacter* spp. (*Enterobacter sakazakii*). A total of 43 *Cronobacter* spp., including 5 clinical isolates, 34 food isolates, 2 environmental isolates, and 2 reference strains (*C. sakazakii* ATCC 29004 and *C. muytjensii* ATCC51329) were used in this study. Korean isolates of *Cronobacter* spp. were divided into 11 biogroups according to their biochemical profiles and 3 genomic groups based on the analysis of their 16S rRNA gene sequences. Biogroups 1 and 2 contained the majority of isolates (n=26), most of which were contained in 16S rRNA cluster 1 (n=34). Korean isolates of *Cronobacter* spp. showed diverse biochemical profiles. Biogroup 1 contained *C. sakazakii* GIHE (Gyeonggi-do Research Institute of Health and Environment) 1 and 2, which were isolated from babies that exhibited symptoms of *Cronobacter* spp. infection such as gastroenteritis, sepsis, and meningitis. Our finding revealed that Biogroup 1, *C. sakazakii*, is more prevalent and may be a more pathogenic biogroup than other biogroups, but the pathogenic biogroup was not represented clearly among the 11 biogroups tested in this study. Thus, all biogroups of *Cronobacter* spp. were recognized as pathogenic bacteria, and the absence of *Cronobacter* spp. in infant foods should be constantly regulated to prevent food poisoning and infection caused by *Cronobacter* spp.

Keywords: *Enterobacter sakazakii*, *Cronobacter* spp., phenotype, 16S rRNA gene cluster

Cronobacter spp. (*Enterobacter sakazakii*) is an opportunistic pathogen belonging to the Enterobacteriaceae family that was initially known as yellow-pigmented *Enterobacter cloacae* and reclassified based on biochemical characteristics, DNA–DNA hybridization, and antibiotic susceptibility patterns in 1980 [5, 18]. *Cronobacter* spp. infection, which causes life-threatening meningitis, septicemia, and necrotizing enterocolitis in infants, has been implicated in foodborne outbreaks worldwide [17, 22]. The pathogen has been isolated from a variety of food, clinical, and environmental sources including cheese, vegetables, grains, soil, and powdered infant formula (PIF) [6, 16, 20]. *Cronobacter* spp. survive dry and osmotic environments [3] and the PIF is contaminated from the processing facilities after pasteurization [4]. In Korea, *Cronobacter* spp. were also isolated from 3 of 45 PIF samples [23], and had contaminated tomato and brown rice used as ingredients for infant foods [12]. Although the mode of transmission of *Cronobacter* spp. has not been clearly identified, infant foods may serve as a vehicle for foodborne transmission. Most reports conducted to date have been greatly concerned with the presence of the foodborne pathogens in infant foods including PIF [2]. To reduce the risk of *Cronobacter* spp. in infant foods, new regulations requiring the absence of *Cronobacter* spp. in 30 samples of every lot were implemented in Europe in 2005 (http://eurlex.europa.eu). Korean regulations requiring the absence of *Cronobacter* spp. in infant foods were implemented in 2007 [15].

Cronobacter spp. strains have been separated into several genetic groups and 16 biogroups [7, 9]. Recently, it was suggested that *E. sakazakii* be reclassified as *Cronobacter* spp. such as *Cronobacter sakazakii*, *Cronobacter malonaticus*, *Cronobacter turicensis*, *Cronobacter dublinensis*, and *Cronobacter muytjensii*, based on the results of biochemical differentiation, 16S rRNA gene sequence analysis, and DNA–DNA hybridization [10, 11]. These results seem to support previous studies that have initially found *Cronobacter*

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spp. to consist of 15 different biogroups based on the diversity of biochemical and genetic characterization [5]. Therefore, it is necessary to evaluate the biochemical and phylogenetic differentiation of Korean isolates of *Cronobacter* spp. isolated from clinics, food, and the environment, and estimate the pathogenicity of each biogroup. The aims of this study were to investigate the phenotypic and genotypic differentiation of Korean isolates of *Cronobacter* spp. and evaluate the pathogenicity of each biogroup.

Materials and Methods

Bacterial Strains

A total of 43 stocked *Cronobacter* spp. including 5 clinical, 34 food, and 2 environmental isolates, and 2 reference strains (*C. sakazakii* ATCC 29004 and *C. muytjensii* ATCC 51329) were evaluated in this study. The 5 clinical isolates were from human feces (GIHE 1~4; Gyeonggi-do Research Institute of Health and Environment, Korea) and diseased part of tympanitis (GIHE 5). The 34 food isolates were from *Sunsik* products and its ingredients. The 2 environmental isolates were from soil. All isolates were stored at −70°C in tryptone soy broth (TSB; Difco, Detroit, MI, USA) with 0.6% yeast extract (Difco) containing 20% glycerol (Difco). All isolates were reactivated by transferring 0.1 ml of the stock culture into 10 ml of TSB and then incubating at 37°C for 24 h. The cultures were streaked onto Druggan−Forsythe−Iversen (DFI; Oxoid, Ltd., Basingstoke, UK) medium and incubated at 37°C for 24 h. Colonies exhibiting a blue-green color during culture on DFI medium were selected for culture on tryptone soy agar (TSA; Difco) at 37°C for 72 h [8]. The colony developing a yellow pigment during culture on TSA [21] was reconfirmed using the Vitek II system with GNI card (bioMèrieix Inc., Marcy l'Etoile, France) and then recultured for 16S rRNA gene amplification and the biochemical testing.

Phenotypic Characterization

Biochemical tests were conducted using conventional manual methods and commercial biochemical kits (ID32E and API 20E, bioMèrieix Inc.). The Voges−Proskauer test was conducted by adding VP1 and VP2 (bioMèrieix Inc.) to Methyl Red Voges−Proskauer Broth (MR-VP; Merck, Darmstadt, Germany) after being cultured at 37°C for 24 h. The Methyl Red test was determined by addition of indicator (0.1 g methyl red per 300 ml of 95% ethanol) to MR-VP Broth cultured at 37°C for 24 h. The reduction of nitrate to nitrite was measured by the addition of NIT1 and NIT2 reagents (bioMèrieix Inc.) to nitrate broth (Difco) cultured at 37°C for 24 h. Motility was determined at 37°C for 48 h using motility test medium (Difco). Acid production from carbohydrates such as myo-inositol, dulcitol, and methyl-α-D-glucopyranoside was tested in purple broth base (Difco) added to each carbohydrate solution at a final concentration 0.5%. Indole production was tested by the addition of James Reagent (bioMèrieix Inc.) to peptone water (Difco) culture incubated at 37°C for 24 h. Malonate utilization was evaluated by culturing the organism in sodium malonate broth (Sigma-Aldrich, St. Louis, MO, USA). Gas production from D-glucose was tested based on the formation of gas bubbles in Durham tubes. All strains were also tested with commercial biochemical kits (ID32E and API 20E; bioMèrieix Inc.) for ornithine utilization. All biochemical kits were used according to the manufacturer's instructions.

Genotypic Characterization

16S rRNA gene partial sequencing was conducted using a modified Sanger method [7, 19]. All isolates were grown on TSB at 37°C for 24 h, after which 1 ml was centrifuged at 13,000 ×*g* for 10 min. The pellet was then washed with 1 ml of sterile distilled water and suspended in 500 µl of sterile distilled water. The cell suspension was boiled for 10 min. After centrifugation at 13,000 ×*g* for 10 min at 4°C, the supernatants were used as templates for PCR. To analyze the 16S rRNA gene partial sequence, the strains were amplified by PCR using primers P0 (5'-AGA GTT TGA TCC TGG CTC AG-3') and P6 (5'-GTA CGG CTA CCT TGT TAC GA-3') [10]. PCR amplification was performed in reaction mixtures with a final volume of 50 µl that contained 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 40 mM KCl, 0.001% gelatin, 250 µM dNTP, 30 pM of each primer, 1 U *Taq* polymerase (Takara Taq, Otsu, Japan), and 10 µl of template DNA. PCR was conducted by subjecting the mixture to the following conditions using a Gene Amp PCR System 9600 (Perkin-Elmer Centus Corp., Foster City, CA, USA): initial denaturation for 3 min at 95°C, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 120 s, and a final cycle at 72°C for 5 min. The PCR products were sequenced by the modified Sanger method using a Big-dye kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3730xl capillary DNA analyzer (Applied Biosystems). A maximum parsimony tree of 16S rRNA gene sequences was developed and analyzed using the Bionumerics software package (version 4.6; Applied Maths, Kortrijk, Belgium). Gaps were not considered an extra state and the topology was optimized using simulated annealing.

Results and Discussion

Biogroups

The biochemical profiles achieved for all isolates including the reference strains were compared with the biogroups originally described by Farmer *et al*. [5]. All isolates were divided into 11 biogroups evaluated by the biochemical tests (Tables 1 and 2). Biogroup 1 contained the majority of isolates (n=15), and all isolates in this group were Voges−Proskauer and ornithine decarboxylase positive. Additionally, these isolates were motile, reduced nitrate, produced gas from D-glucose, and produced acid from myo-inositol, dulcitol, and methyl-α-D-glucopyranoside. Biogroup 2 was negative for acid production from inositol (n=11). Biogroup 3 was nonmotile and only contained the *C. sakazakii* ATCC 29004 reference strain. Biogroup 4 was represented by only one isolate, which was negative for ornithine decarboxylase, and Biogroup 5 was positive for malonate utilization (n=5). Biogroup 6 was positive for indole (n=2) and Biogroup 9 (inositol negative) contained two isolates. Biogroup 10 (n=2) was inositol negative and indole positive. Biogroup 11 was represented by only one isolate that was inositol negative and dulcitol positive. Biogroup 12 was indole and

Table 1. Sources of Korean isolates of *Cronobacter* spp. (*Enterobacter sakazakii*) used in this study.

Farmer biogroup	Isolation source				Total
	Clinical[a]	Food[b]	Environmental[c]	Reference strain[d]	
1	3	12			15
2	1	10			11
3				1	1
4		1			1
5		4	1		5
6		2			2
9	1	1			2
10		2			2
11		1			1
12		1	1		2
15				1	1
Total	5	34	2	2	43

[a]The clinical isolates were isolated from human feces (GIHE 1~4; Gyeonggi-do Research Institute of Health and Environment) and from the diseased part of tympanitis (GIHE 5).
[b]The food isolates were isolated from *Sunsik* products and its ingredients.
[c]The environmental isolates were isolated from soil.
[d]*Cronobacter sakazakii* ATCC 29004 (Farmer Biogroup 3) and *Cronobacter muytiensii* ATCC 51329 (Farmer Biogroup 15).

Table 2. Assignment of Korean isolates of *Cronobacter* spp. (*Enterobacter sakazakii*) to the biogroups originally defined by Farmer *et al*. [5].

Farmer biogroup	Phenotype[a]											No. of strains
	VP	MR	Nit	Orn	Mot	Ino	Dul	Ind	Malo	Gas	Glu	
1	+	-	+	+	+	+	-	-	-	+	+	15
2	+	-	+	+	+	-	-	-	-	+	+	11
3	+	-	+	+	-	+	-	-	-	+	+	1[b]
4	+	-	+	-	+	+	-	-	-	+	+	1
5	+	-	+	+	+	+	-	-	+	+	+	5
6	+	-	+	+	+	+	-	+	-	+	+	2
9	+	-	+	+	+	-	-	-	+	+	+	2
10	+	-	+	+	+	-	-	+	-	+	+	2
11	+	-	+	+	+	-	+	-	-	+	+	1
12	+	-	+	+	+	+	-	+	+	+	+	2
15	+	-	+	+	+	+	+	+	+	+	+	1[c]

[a]VP, Voges–Proskauer; MR, methyl red; Nit, nitrate reduction; Orn, ornithine utilization; Mot, motility at 37°C; Ino, acid production from inositol; Dul, acid production from dulcitol; Ind, indole production; Malo, malonate utilization; Gas, gas production from glucose; Glu, acid production from α-methyl-D-glucopyranoside.
[b]*Cronobacter sakazakii* ATCC 29004.
[c]*Cronobacter muytiensii* ATCC 51329.

malonate positive (n=2). Biogroup 15 only contained the *C. muytjensii* ATCC 51329 reference strain, which was positive for all tests except methyl red. When the species were defined by Farmer *et al*. [5], 15 biogroups of *Cronobacter* spp. were described according to the biochemical profiles, and Biogroup 16, a new biogroup, was defined by Iversen *et al.* [9]. Recently, it has been suggested that *E. sakazakii* should be reclassified as *Cronobacter* spp. based on biochemical differentiation [10, 11]. *C. sakazakii* (Biogroups 1, 2, 3, 4, and 11) contained 29 isolates including 4 clinical, 24 food, and 1 reference strain (*C. sakazakii* ATCC 29004). *C. malonaticus* (Biogroups 5 and 9) contained 7 isolates including 1 clinical, 5 food, and 1 environmental isolate. *C. dublinensis* (Biogroups 6, 10, and 12) contained 6 isolates including 5 food and 1 environmental isolate. *C. muytjensii* (Biogroup 15) only contained the *C. muytjensii* ATCC 51329 reference strain. In the present study, all isolates including the reference strain were divided into 11 biogroups, which was in agreement with the results of previous studies [5, 9], and Korean isolates of *Cronobacter* spp. tested in this study were found to express a variety of biochemical characteristics. Biogroups 1 and 2 contained the majority of the strains (n=26), and *C. sakazakii* (n=29) was the most frequent strain among *Cronobacter* spp.

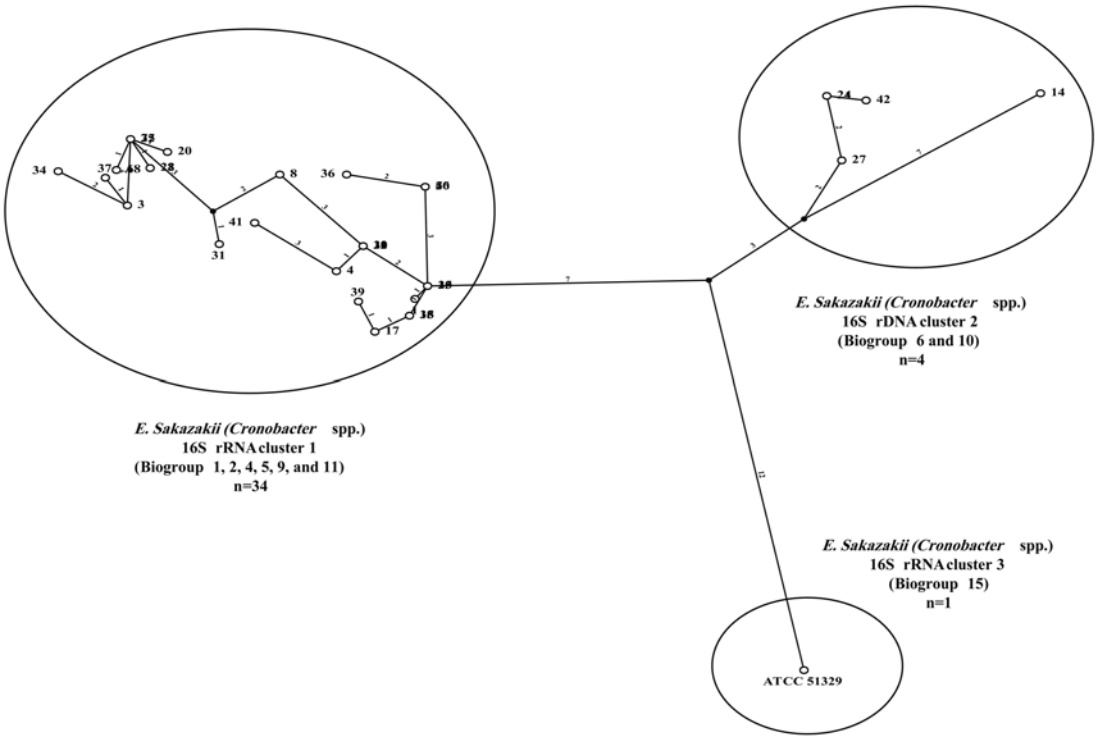

Fig. 1. Maximum parsimony tree (unrooted) showing the three genomic clusters of Korean isolates of *Cronobacter* spp. (*Enterobacter sakazakii*) based on 16S rRNA partial gene sequence analysis.
Gaps were not considered an extra state, and the topology was optimized using simulated annealing.

Five clinical isolates tested in this study belonged to three biogroups according to biochemical differentiation (Tables 1 and 2). Biogroup 1 contained *C. sakazakii* GIHE (Gyeonggi-do Research Institute of Health and Environment) 1, 2, and 5, which were isolated from a 3-month-old baby who exhibited symptoms of gastroenteritis and meningitis, a 20-day-old baby who presented with sepsis and meningitis, and a 5-year-old boy who did not show any significant symptoms, respectively [13]. Biogroup 2 consisted of *C. sakazakii* GIHE 4 isolated from a 72-year-old male, and Biogroup 9 contained *C. malonaticus* GIHE 3 isolated from a 64-year-old male, neither of whom exhibited significant symptoms. These results indicated that Biogroup 1, *C. sakazakii*, is more prevalent and may be a more pathogenic biogroup than the other biogroups, but the pathogenic biogroup was not represented clearly among the 11 biogroups. Thus, all biogroups of *Cronobacter* spp. were recognized as pathogenic bacteria, and the absence of *Cronobacter* spp. in infant foods should be constantly regulated to prevent food poisoning and infection caused by *Cronobacter* spp.

Phylogenetic Analysis

The partial 16S rRNA gene sequences (>1,017 bases) were analyzed using the maximum parsimony method; however, four isolates (GIHE 19, 21, 29, and *C. sakazakii* ATCC 29004) were excluded from these analyses because the recovered 16S rRNA sequences were too short (<500 bases). The 16S rRNA gene is highly conserved and ubiquitous, and the gene is widely used for sequence analysis to differentiate species [14]. The maximum parsimony method is a simple and popular technique for construction of phylogenetic trees. This method is an evolutionary model that uses the fewest inferred changes between characters [1, 9]. In the present study, 39 isolates, including a reference strain, were divided into 3 genomic groups by 16S rRNA gene sequence analysis (Fig. 1). 16S rRNA cluster 1 contained Biogroups 1, 2, 4, 5, 9, and 11 originally defined by Farmer III *et al*. [5]. The majority of the strains used in this study were contained in this cluster (n=34). 16S rRNA cluster 2 included Biogroups 10 and 12 (n=4), and 16S rRNA cluster 3 was composed of Biogroup 15, which only included *C. muytjensii* ATCC 51329 (Table 3). *C. sakazakii* and *C. malonaticus* were present in 16S rRNA cluster 1. *C. dublinensis* and *C. muytjensii* were present in 16S rRNA clusters 2 and 3, respectively. These findings were in agreement with those of a previous report in which *Cronobacter* spp. were divided into four genomic groups by 16S rRNA gene sequence analysis and each 16S rRNA cluster contained the same biogroups [9]. Korean isolates of *Cronobacter* spp. including the reference strain showed three genomic groups and clinical strains that were present in 16S rRNA

cluster 1. These results revealed that 16S rRNA cluster 1 is the more prevalent and pathogenic cluster among Korean isolates of *Cronobacter* spp., because all clinical isolates, especially GIHE1 and 2, which were isolated from babies exhibiting symptoms of gastroenteritis, sepsis, and meningitis, were contained in this cluster. Thus, the 16S rRNA cluster 1 of *Cronobacter* spp. was handled with more care than the other clusters.

Table 3. Biogroups and 16S rRNA clusters of Korean isolates of *Cronobacter* spp. (*Enterobacter sakazakii*).

Strain No.	Isolated source	Farmer biogroups	16S rRNA cluster	*Cronobacter* spp.
GIHE [a] 1	Clinical	1	1	*Cronobacter sakazakii*
GIHE 2	Clinical	1	1	
GIHE 5	Clinical	1	1	
GIHE 10	Food	1	1	
GIHE 13	Food	1	1	
GIHE 15	Food	1	1	
GIHE 16	Food	1	1	
GIHE 18	Food	1	1	
GIHE 20	Food	1	1	
GIHE 22	Food	1	1	
GIHE 23	Food	1	1	
GIHE 26	Food	1	1	
GIHE 28	Food	1	1	
GIHE 34	Food	1	1	
GIHE 36	Food	1	1	
GIHE 4	Clinical	2	1	
GIHE 6	Food	2	1	
GIHE 8	Food	2	1	
GIHE 11	Food	2	1	
GIHE 12	Food	2	1	
GIHE 17	Food	2	1	
GIHE 25	Food	2	1	
GIHE 30	Food	2	1	
GIHE 33	Food	2	1	
GIHE 38	Food	2	1	
GIHE 40	Food	2	1	
Cronobacter sakazakii ATCC29004		3	ND [b]	
GIHE 37	Food	4	1	
GIHE 39	Food	11	1	
GIHE 7	Food	5	1	*Cronobacter malonaticus*
GIHE 19	Food	5	ND	
GIHE 32	Food	5	1	
GIHE 35	Food	5	1	
GIHE 41	Environmental	5	1	
GIHE 3	Clinical	9	1	
GIHE 31	Food	9	1	
GIHE 21	Food	6	ND	*Cronobacter dublinensis*
GIHE 29	Food	6	ND	
GIHE 14	Food	10	2	
GIHE 27	Food	10	2	
GIHE 24	Food	12	2	
GIHE 42	Environmental	12	2	
Cronobacter muytiensii ATCC51329		15	3	*Cronobacter muytiensii*

[a]Gyeonggi-do Research Institute of Health and Environment. [b]Not determined.

In conclusion, Korean isolates of *Cronobacter* spp. including the reference strains were divided into 11 biogroups according to the biochemical profiles, and 3 genomic groups based on the 16S rRNA gene sequence analysis. Korean isolates of *Cronobacter* spp. showed diverse biochemical profiles. Biogroups 1 and 2 contained the majority of the strains (n=26), and most of these fell into 16S rRNA cluster 1 (n=34). Biogroup 1, *C sakazakii*, may be more prevalent than other biogroups, but the pathogenic biogroup was not clearly represented among the 11 biogroups. Thus, all biogroups of *Cronobacter* spp. were recognized as pathogenic bacteria, and the absence of *Cronobacter* spp. in infant foods should be constantly regulated to prevent food poisoning and infection caused by *Cronobacter* spp.

References

1. Barker, D. 2004. LVB: Parsimony and simulated annealing in the search for phylogenetic trees. *Bioinformatics* **20:** 274–275.
2. Biering, G., S. Karlsson, N. C. Clark, K. E. Jónsdóttir, and P. Lúdvígsson. 1989. Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. *J. Clin. Microbiol.* **27:** 2054–2056.
3. Breeuwer, P., A. Lardeau, M. Peterz, and H. M. Joosten. 2003. Desiccation and heat tolerance of *Enterobacter sakazakii*. *J. Appl. Microbiol.* **95:** 967–973.
4. Drudy, D., M. O'Rourke, M. Murphy, N. R. Mullane, R. O'Mahony, L. Kelly, *et al*. 2006. Characterization of a collection of *Enterobacter sakazakii* isolates from environment and food sources. *Int. J. Food Microbiol.* **110:** 127–134.
5. Farmer III, J. J., M. A. Asbury, F. W. Hickman, Don J. Brenner, and The Enterobacteriaceae Study Group. 1980. *Enterobacter sakazakii*: A new species of "Enterobacteriaceae" isolated from clinical specimens. *Int. J. Syst. Bacteriol.* **30:** 569–584.
6. Iversen, C. and S. Forsythe. 2003. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. *Trends Food Sci. Technol.* **14:** 443–454.
7. Iversen, C., M. Waddington, S. L. On, and S. Forsythe. 2004. Identification and phylogeny *of Enterobacter sakazakii* relative to *Enterobacter* and *Citrobacter* species. *J. Clin. Microbiol.* **42:** 5368–5370.
8. Iversen, C., P. Druggan, and S. Forsythe. 2004. A selective differential medium for *Enterobacter sakazakii*, a preliminary study. *Int. J. Food Microbiol.***96:** 133–139.
9. Iversen, C., M. Waddington, J. J. Farmer III, and S. J. Forsythe. 2006. The biochemical differentiation of *Enterobacter sakazakii* genotypes. *BMC Microbiol.* **6:** 94–100.
10. Iversen, C., A. Lehner, N. Mullane, E. Bidlas, I. Cleenwerck, J. Marugg, S. Fanning, R. Stephan, and H. Joosten. 2007. The taxonomy of *Enterobacter sakazakii*: Proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter* 1. *BMC Evol. Biol.* **7:** 64–74.
11. Iversen, C., N. Mullane, B. McCardell, B. D. Tall, E. Bidlas, A. Lehner, S. Fanning, R. Stephan, and H. Joosten. 2008. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter* genomospecies 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. *Int. J. Syst. Evol. Microbiol* **58:** 1442–1447.
12. Jung, M. K. and J. H. Park. 2006. Prevalence and thermal stability of *Enterobacter sakazakii* from unprocessed ready-to-eat agricultural products and powdered infant formulas. *Food Sci. Biotechnol.* **15:** 152–157.
13. Kim, J. B., S. H. Cho, Y. B. Park, J. B. Lee, J. C. Kim, B. K. Lee, H. K. Lee, and H. S. Chae. 2008. Surveillance of stool samples for the presence of *Enterobacter sakazakii* among Korean people. *Yonsei Med. J.* **49:** 1017–1022.
14. Kolbert, C. P. and D. H. Persing. 1999. Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. *Curr. Opin. Microbiol.* **2:** 299–305.
15. Korea Food and Drug Administration. 2008. KFDA. Korea Food Code. P. 10-3-43. Seoul, Korea.
16. Leclercq, A., C. Wanegue, and P. Baylac. 2002. Comparison of fecal coliform agar and violet red bile lactose agar for fecal coliform enumeration in foods. *Appl. Environ. Microbiol.* **68:** 1631–1638.
17. Lehner, A. and R. Stephan. 2004. Microbiological, epidemical, and food safety aspects of *Enterobacter sakazakii*. *J. Food Prot.* **67:** 2850–2857.
18. Nazarowec-White, M. and J. M. Farber. 1997. *Enterobacter sakazakii*: a Review. *Int. J. Food Microbiol.* **34:** 103–113.
19. Sanger, F., S. Nicklen, and A. R. Coulson. 1992. DNA sequencing with chain-terminating inhibitors. 1977. *Biotechnology* **24:** 104–108.
20. Simmons, B. P., M. S. Gelfand, M. Haas, L. Metts, and J. Ferguson. 1989. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of a powdered infant formula. *Infect. Cont. Hosp. Epidemiol.* **10:** 398–401.
21. US Food and Drug Administration. 2002. Isolation and enumeration of *Enterobacter sakazakii* from dehydrated powdered infant formula. Available at http://www.cfsan.fda.gov/~comm/mmesakaz.html. Accessed 10 December 2009.
22. Van Acker, J., F. de Smet, G. Muyldermans, A. Bougatef, A. Naessens, and S. Lauwers. 2001. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. *J. Clin. Microbiol.* **39:** 293–297.
23. Yoo, M. K., S. S. Kim, and S. S. Oh. 2005. Isolation and genotyping of *Enterobacter sakazakii* from powdered infant formula manufactured in Korea. *Food Sci. Biotechnol.* **14:** 875–877.