Note

Occurrence of *Cronobacter* spp. in Dried Foods, Fresh Vegetables and Soil

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The present study surveyed the occurrence of *Cronobacter* spp. in dried foods including milk powder, spices and herbs and others, and fresh vegetables commercially available in markets, and ground soil materials for the agriculture. *Cronobacter* spp. were isolated from 15% of 33 spice and herb samples and 3% of 36 taste foods, and these were *C. turicensis*, *C. malonaticus*, *C. sakazakii* and *C. dubliensis*. *Cronobacter* spp. from fresh vegetables were detected in 12% of field vegetables and 13% of hydroponic vegetables. *C. turicensis* was prevalent in field vegetables, and *C. malonaticus* was in hydroponic ones. And, *Cronobacter* spp. in shredded vegetables were detected from 44% of 9 samples, and these were *C. dubliensis*, *C. turicensis* and *C. sakazakii*. Also, *Cronobacter* spp. in soil from rice field, vegetable field and sandpits were predominantly *C. sakazakii* and *C. malonaticus*.

Key words : *Cronobacter* spp. / Dried food / Fresh vegetables / Soil.

Cronobacter spp. are recognized as opportunistic pathogens causing rarely life-threatening infections such as meningitis, necrotizing enterocolitis and bacteremia in premature, low-birth and immunocompromised infants (FAO/WHO, 2008). Particularly, it should be paid attention that infants given contaminated milk formula suffered from meningitis, enteritis and sepsis (Simmons et al., 1989; Van Acker et al., 2001). Therefore, the bacteria should be considered as one of agents of foodborne illnesses.

Cronobacter spp. are gram-negative, facultative anaerobic, nonspore-forming, motile bacteria belonging to the family Enterobacteriaceae. Iversen et al., (2008) described that the species consisted of *S. sakazakii*, *C. dublinensis*, *C. dublinensis* subsp. *dublinensis*, *C. dublinensis* subsp. *lactaridi*, *C. dublinensis* subsp. *lausannensis*, *C. malonaticus*, *C. muytjensii*, *C. turicensis* and the unnamed genotype species I. Thereafter, *C. condimenti* and *C. universalis* were added in 2012 (Joseph et al., 2012), and *C. helveticus*, *C. pulveris* and *C. zurichensis* were added in 2013 (Brady et al., 2013). The natural habitat of these bacteria is unknown, but the various growth properties suggests that *Cronobacter* spp. are associated with plants (Schmid et al., 2009).

Cronobacter spp. have been isolated in various environmental materials such as soil, water, dust from household and milk powder factories, and utensils used to mix infant formula (Neelam et al., 1987; Friedemann, 2007, Kndhai et al., 2004; Muytjens et al., 1983). Moreover, *Cronobacter* spp. have been isolated from a wide spectrum of foods and food ingredients (Friedmann, 2007). The present survey aimed to investigate the occurrence of *Cronobacter* spp. in dried foods including milk powder, spices and herbs and others, and fresh vegetables commercially available in markets, and ground soil materials for the agriculture, and the bacterial isolates were identified by means of standard biochemical tests.

A total of 228 samples of dried foods were purchased from retailers in Kantoh area. Their survey was examined on 33 kinds of dried spices and herbs, 64 of powdered milk products, 11 of dried fruits, 7 of sea food products, 36 of dried taste foods, 49 of convenience foods and 28 of other dried type-foods. Also, a total of 123 samples of fresh vegetables obtained from retailers in Saitama prefecture. The fresh vegetables were 60 samples harvested from outdoor fields, 54 samples cultured hydroponically and 9 samples of shredded vegetable. In addition, soil samples were collected from 25 of rice fields, 26 of vegetable fields, 24 of flower beds and 25 of park sandpits in Saitama

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prefecture.

The detection and isolation of *Cronobacter* spp. were performed according to the procedure described in the directive CSN P ISO/TS 22964 (2006) Milk and milk products – Detection of *Enterobacter sakazakii*. Briefly, aliquots (5 g or 10 g) of each of tested samples were weighed separately and aseptically into sterile stomacher bags, diluted with 45 or 90 ml of buffered peptone water (Oxoid, UK), and homogenized in a stomacher for 2 min. The homogenate was incubated at 37°C for 18 h. Then, 0.1 ml of the pre-enriched sample was transferred into 10 ml of Lauryl Sulfate Broth (Oxoid, UK) supplemented with NaCl to a final concentration of 34 mg/ml and vancomycin to a final concentration of 0.01 mg/ml. The sample was incubated at 44°C for 24 h. One loop of the selectively enriched culture was streaked onto the surface of Chromocult Enterobacter Sakazakii Agar (ESA; Merck, Germany) and incubated at 44°C for 24 h. Green or blue-green colonies which were termed presumptive *Cronobacter* spp. were streaked onto Trypticase Soy Agar (Difco, USA) to make a pure culture. After incubation at 25°C for 48 h, the purity of

the culture could be checked by confirming that all colonies were yellow. Their identification was by means of biochemical tests. In addition to Gram staining and phenotype characterization derived from commercial API ID 32 E (bioMeriux CZ, Czech Republic) devices according to procedure's instructions, the following tests were performed using conventional manual methods: motility test, methyl red test, reduction of nitrate, gas formation from D-glucose, production of catalase, cytochrome oxidase test, indole production, utilization of malonate, and acid production from dulcitol.

The standard plate counts and the qualitative detection results of coliform bacteria and *Cronobacter* spp. from dried foods, fresh vegetables and soil samples were shown in Table 1. The average plate counts in dried foods were 2.1 ± 1.2 Log CFU/g, and the counts were higher in spices and herbs, and sea foods than other dried food. Coliforms were detected only in 1 (14%) sample among seafood samples. *Cronobacter* spp. were detected from 5 (15%) of 33 kinds of spices and herbs and 1 (3%) of 36 taste foods. The detection frequency of *Cronobacter* spp. was especially high in herbs and

TABLE 1. Incidences of standard plate counts, coliform bacteria, *Cronobacter* spp. in dried foods, fresh vegetables and soil samples.

	No. of samples	SPC ¹⁾ Log CFU/g	No. (%) of positive samples:	
			Coliforms ²⁾	<i>Cronobacter</i> spp. ³⁾
Dried foods				
Spices and Herbs	33	3.0±1.4		5 (15%)
Powdered milk	64	1.2±0.4		
Dried fruits	11	1.5±0.7		
Sea foods	7	3.8±1.1	1 (14%)	
Dried taste foods	36	1.5±0.5		1 (3%)
Convenience foods	49	2.0±0.8		
Other	28	2.0±1.1		
Total	228	2.1±1.2	1 (0.4%)	6 (2.6%)
Fresh vegetables				
from Fields	60	3.6±0.9	38 (63%)	7 (12%)
Hydroponic cutures	54	4.7±0.4	37 (69%)	7 (13%)
Shredded vegetables	9	4.5±0.1	8 (89%)	4 (44%)
Soil				
Rice fields	25	6.0±0.2	5 (20%)	1 (4%)
Vegetable fields	26	6,1±0.3	4 (15%)	4 (15%)
Flower beds	25	5.9±0.2	10 (40%)	
Sandboxes	24	5.0±0.7	7 (29%)	8 (33%)

1) SPC: Standard plate counts

2) Coliforms were estimated by conventional MPN assay with 5.55 g of samples.

3) *Cronobacter* spp. were detected after 5 or 10g of samples were precultured in buffered peptone water 37°C for 18 h.

TABLE 2. Occurrence of *Cronobacter* spp. in dried food, fresh vegetable and soil samples.

Sample	No. of positives	No. of <i>Cronobacter</i> strains ¹⁾						Unident. ²⁾
		Total	Cs	Cm	Cd	Ct		
Dried foods								
Peppermint	1	10	4	6				
Basil	1	11	5	3	3			
Italian parsley	1	7	5				2	
Marjoram	1	20	1	4			15	
Sage	1	6		1			5	
Total	5	54	15	14	3		22	
creaming powder	1	8		3			5	
Fresh vegetables								
Field vegetables								
Spinach	1	4					4	
Carrot	1	3					3	
Turnip	1	1	1					
		1	2				2	
Okra	3	2	1				1	
		3	2					2
Cherry tomatoes	1	1				1		
Total	7	14	1			1	10	2
Hydroponic cultures								
		1	3		3			
Leaf carrot	3	2	3	1	2			
		3	3		3			
Alfalfa	1	1	3	3				
		1	1	1				
Soybean sprouts	3	2	1		1			
		3	1					1
Total	7		15	5	9			1
Shredded vegetable mixtures								
		1	4		4			
	4	2	3				3	
		3	3	3				
		4	1				1	
Total			11	3	4		4	
Soil								
Rice field	1		2		2			
		1	12	1	10	1		
Vegetable field	4	2	5		3	2		
		3	3	1	2			
		4	9	3	6			
Total			29	5	21	3		
		1	15	11	3	1		
		2	2	2				
		3	2	2				
Sandboxes	8	4	4	3			1	
		5	10	10				
		6	4		4			
		7	1		1			
		8	1		1			
Total			39	28	9	1	1	

1) Cs: *C. sakazakii*; Cm: *C. malonaticus*; Cd: *C. dublinensis*; Ct: *C. turicensis*

2) Unident.: Unidentification

spices, but other 222 dried food samples except 1 taste food (creaming powder) were not contaminated with *Cronobacter* spp. On fresh vegetables, the average standard plate counts were 3.6 ± 0.9 Log CFU/g in ones from fields, and 4.7 ± 0.4 Log CFU/g in hydroponic cultures, while coliforms were detected in 38 (63%) among 60 of field vegetables, and in 37 of 54 (69%) hydroponic vegetables. Also, *Cronobacter* spp. were detected in 7 (12%) of field vegetables and 7 (13%) of hydroponic vegetables. In commercial artificially shredded vegetables, the average plate counts were 4.5 ± 0.1 Log CFU/g, coliforms were detected in 8 of 9 (89%) samples, and *Cronobacter* spp. were isolated from 4 of 9 (44%) samples. On soil samples, the standard plate counts were 6 Log CFU/g in rice fields, vegetable fields and flower beds, but 5 Log CFU/g in the samples from sandboxes. Coliforms from soil samples were detected in 5 of 25 (20%) rice field samples, 4 (15%) of 26 vegetable field ones, 10 of 25 (40%) flower bed ones, and 7 of 24 (29%) sandbox ones. Also, *Cronobacter* spp. were isolated from 1 (4%) of rice field samples, 4 (15%) of vegetable field ones, and 8 (33%) of sandbox ones, but they were not detected from all flower bed samples. Ogihara et al. (2014) suggested that there were positive correlations between the viable-coliforms counts and *Cronobacter* counts as reported by Jung and Park (2006). But, samples contaminated with *Cronobacter* were not necessarily contaminated with coliforms and high levels of the plate counts.

The species of *Cronobacter* spp. isolated from dried food, fresh vegetable and soil samples were shown in Table 2. Among dried foods, *Cronobacter* spp. were isolated from 5 kinds of spices and herbs containing peppermint, basil, Italian parsley, marjoram and sage, and creaming powder. The identified *Cronobacter* spp. were *C. turicensis* (44%), *C. malonaticus* (27%), *C. sakazakii* (24%) and *C. dubliensis* (5%). Five kinds of fresh vegetables (spinach, carrot, turnip, okra and cherry tomatoes) from fields were contaminated with *C. turicensis* (71%), *C. sakazakii* (7%) and *C. dubliensis* (7%). Leaf carrot, alfalfa and soybean sprouts among vegetables cultured hydroponically were contaminated with *C. malonaticus* (60%) and *C. sakazakii* (33%). Also, *C. dubliensis* (36%), *C. turicensis* (36%) and *C. sakazakii* (27%) were isolated from 4 commercially shredded vegetable samples. Although Hochel et al. (2012) and Ogihara et al. (2014) surveyed dried foods and food materials other than dried foods on incidences of *Cronobacter* spp., of which *C. sakazakii* was the most common contaminant, and some differences were recognized every food samples as for the distribution of *Cronobacter* species. On soil materials, *Cronobacter* spp. from 1 rice field was *C. malonaticus* only, whereas the common species from 4 vegetable fields were *C.*

malonaticus (74%) and *C. sakazakii* (17%), and the common species from 8 sandboxes in parks were *C. sakazakii* (72%) and *C. malonaticus* (23%). In the present survey, the members of *Cronobacter* spp. were different among material types, and *Cronobacter* members were not necessarily found between dried foods, fresh vegetables and soil materials.

The members of *Cronobacter* spp. are ubiquitous bacteria that have been found in various foods particularly of plant origin and dried material. Some spice and herb materials and fresh vegetables tested in the present survey were contaminated with low level of *Cronobacter* spp. Although an impact of the consumption of contaminated foods on infants, elderly or immunocompromised individuals is not sufficient, addition of spices and herbs after cooking or handling of fresh vegetables should be noted in kitchen since *Cronobacter* spp. can multiply at room temperature, and conventional hygienic handling of foods would be required to control the viable bacterial counts, especially of *Cronobacter* spp.

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