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Cronobacter sakazakii in baby foods and baby food ingredients of dairy origin and microbiological profile of positive samples

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ABSTRACT

Cronobacter sakazakii is an opportunistic food-borne pathogen isolated from powdered infant formula (PIF), follow-up formula (FUF) and a variety of foods. Two different methods were used for the isolation of *Cronobacter* spp. from baby food ingredients, PIF and FUF in this study. Each presumptive *C. sakazakii* isolate was then subjected to identification by polymerase chain reaction (PCR) with partial 16S rRNA gene sequencing. The prevalence of *C. sakazakii* was 7.5% and the number of *C. sakazakii* was between 7 and 15 MPN/g in milk and whey powders. *Cedecea lapagei* was detected in PIF for the first time in this study. Other pathogenic bacteria such as *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. were not detected in the samples. *Cedecea lapagei* is also an opportunistic pathogen and was found in PIF and thus, care must be taken and effective control measures should be applied in the production of PIF.

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1. Introduction

Cronobacter sakazakii is an emerging pathogen which causes infections in infants, children and adults especially the elderly and immunocompromised adults. The disease is more serious among newborns or young infants, with mortality rates of 40–80% worldwide (Friedemann, 2009; Nazarowec-White & Farber, 1997). It has been implicated in severe forms of neonatal infections such as meningitis, bacteraemia, sepsis, and necrotizing enterocolitis (Kleiman, Allen, Neal, & Reynolds, 1981). Although *Cronobacter* spp. have been mainly associated with infections in infants, recent data from the Foodborne Diseases Active Surveillance Network have shown that the number of infected adults of >70 years of age (37%) were higher than the number of infected infants of <1 year of age (4%) in the United States (Patrick et al., 2014). In adults, *Cronobacter* spp. may cause pneumonia, septicemia, osteomyelitis, splenic abscesses, and wound infections (Healy et al., 2010). The incident rates of invasive infection (cases per100,000 population) varied by age and were highest among infants of <1 year of age (0.49) and persons >80 years of age (0.33) (Patrick et al., 2014).

C. sakazakii has common characteristics similar to that of other members of Enterobacteriaceae such as being Gram-negative, non-spore-forming, and facultative anaerobic. It is motile with a peritrichous flagella, and can be recognized as typical yellow pigmented colonies on Tryptic Soy Agar (TSA). This bacterium was known as *Enterobacter sakazakii* formerly, but has been reclassified as *C. sakazakii* as described by Iversen et al., (2008).

Recently, new species were added to the genus *Cronobacter* based on molecular characterization [by using multilocus sequence typing (MLST)] (Baldwin et al., 2009), [sequence types (STs)] (Iversen et al., 2008), [as well as single nucleotide polymorphisms] (Stoop, Lehner, Iversen, Fanning, & Stephan, 2009). Based on (this recent identification) the molecular identification of the genus *Cronobacter* consists of seven species: *C. condimenti*, *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C. sakazakii*, *C. turicensis*, and *C. universalis*, (Iversen et al., 2008; Joseph, Hariri, & Forsythe, 2013; Joseph et al., 2012; Masood et al., 2013; Yan et al., 2012). Among them *C. sakazakii*, *C. malonaticus* and *C. turicensis* are the most frequently isolated from infected infants (Joseph & Forsythe, 2011). *C. zurichensis*, *C. helveticus* and *C. pulveris* are the recently reclassified species (Zimmermann, Schmidt, Loessner, & Weiss, 2014).

Contaminated powdered infant formula was epidemiologically linked with infant infections (Himelright, Harris, Lorch, Anderson, & Jones, 2002; Teramoto, Tanabe, Okano, Nagashima, & Kobayashi, 2010). Studies also show that the possible reservoirs of *Cronobacter* spp. (infant infections) was powdered infant formula (PIF) as well as other baby foods (Biering, Karlsson, Clark, Jonsdottir, & Ludvigsson, 1989; Yan et al., 2012). However, it was also reported

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that extrinsic contamination from the surrounding environment can occur and unintentionally human beings can be a carrier and may contaminate the product (Jaradat, Al Mousa, Elbetieha, Al Nabulsi, & Tall, 2014).

Raw ingredients of different origin and/or thermally sensitive ingredients added to PIF without any prior heat treatment have been implicated as a potential route of transmission for the bacterium (Healy et al., 2010). *Cronobacter* spp. were isolated from dairy products e.g. cheese (Chaves-Lopez, De Angelis, Martuscelli, Serio, & Paparella, 2006); mixed vegetables and salads (Osaili & Forsythe, 2009); spices (Belal, Al-Mariri, Hallab, & Hamad, 2013) and number of other foods (Beuchat, Kim, Gurtler, Lin, & Ryu, 2009). Since the infections in children, adult and elderly have also been reported (Healy et al., 2010), retail foods (e.g. dried milk and whey powder, as well as other milk products e.g. cheese) might be the most likely to harbor this opportunistic pathogen. Therefore, the aim of the study was to investigate milk based ingredients such as milk powder, whey powder, and cheese as well as powdered infant formula and follow-up formula for the presence of *C. sakazakii*. The microbiological profile of positive samples was also determined to better explain the hygienic quality of the samples. Since standard and rapid microbiological methods were used for the determination and identification of *Cronobacter* spp., in this study, 16 rRNA method was also applied for confirmation of *C. sakazakii* strains.

2. Material and methods

2.1. Material

In this study, 114 samples of milk powder (25), whey powder (15), cheese, (19), powdered infant formula (40), and follow-up formula (15) were studied for the presence of *Cronobacter sakazakii*. These products are either used as ingredients for infant/baby foods or consumed by human beings. Powdered samples were obtained from the producer directly in their original packages and cheese samples were purchased from markets in their original packages as well.

2.2. Cronobacter sakazakii analysis

Two different methods were applied for the isolation of *Cronobacter* from the samples. An ISO/IDF (2006) method was followed as a first method. In this method, 225 mL buffered peptone water was used to pre-enrich samples (25 g) and incubated at 37 °C for 18 h. For the re-enrichment, 0.1 mL enriched sample was added to 10 mL modified lauryl sulfate tryptose (mLST) broth (20.0 g tryptose, 5.0 g lactose, 5.0 g sodium chloride, 2.75 g dipotassium hydrogen phosphate, 2.75 g potassium dihydrogen phosphate, 0.1 g sodium lauryl sulfate, and 10 mg/L vancomycin) and incubated at 44 ± 0.5 °C for 24 h. Then, Chromogenic agar [Oxoid, UK CM 1055 DFI (Druggan, Forsythe, Iversen)- Chromogenic *E. sakazakii* agar] was used and plates were incubated at both 44 ± 0.5 °C for 24 h and 37 ± 0.5 °C for 24 h for specific detection of *Cronobacter* species. Colonies that appeared blue-green on DFI agar plates were then streaked onto tryptic soy agar (TSA, Oxoid, UK CM0131) for reconfirmation. Finally, colonies appearing yellow on TSA agar plates were used for biochemical tests using API20E (Biomerieux, France) identification kits.

BAX® System PCR assay designed for *C. sakazakii* was used as a second method. Qualicon BAX® System Q7 (DuPont, Wilmington, USA) was used and the protocol developed by the producer was followed. Briefly, 225 mL mLST was used to pre-enrich samples (25 g) and incubated at 45 ± 1 °C for 22 h. Enriched sample (10 μL) was added to 500 μL brain heart infusion broth (BHI, Oxoid, UK) and incubated at 37 ± 0.5 °C for 3 h. Protease (150 μL) mixed with lysis buffer and transferred 200 μL of lysis reagent to cluster tubes. Samples (5 μL; from BHI) transferred to cluster tubes. Cluster tubes were heated at 37 ± 2 °C for 20 min, and then at 95 ± 3 °C for 10 min. Cluster tubes were cooled for 5 min in a cooling block, then 50 μL transferred to PCR tubes in a cooling block. Sealed PCR tubes were placed in cycler and the program was run. The results were obtained as positive or negative.

All cultures were subjected to identification by polymerase chain reaction (PCR) with partial 16S rRNA gene sequencing. In this study, partial 16S rRNA gene sequencing was used with the universal primers: forward 5′ CCG TCA ATT CCT TTG AGT TT 3′ and reverse 3′ AGA GTT TGA TCC TGG CTC AG 5′. The resulting PCR product was sequenced; and sequence similarity search was carried out with BLAST (Basic Local Alignment Search Tool). Comparisons of reference strain and isolates sequences were performed using Clustal W alignment algorithm. The dendogram constructed (Neighbor-joining) from the partial 16S sequences of isolates and matching sequences from the reference strains found in databases (NCBI gene bank) is presented in Fig. 1. Their accession numbers are as follows: *C. sakazakii* (800), *C. dublinensis* (22) (KF360300) and *Cedecea lapagei* (37–10).

2.3. Enumeration of Cronobacter sakazakii

The FDA (2002) method was used for the enumeration of *C. sakazakii* in the samples. Samples (1, 10, and 100 g) were weighed in triplicate and transferred to 125 mL, 250 mL, and 2 L size sterile Erlenmayer flasks, respectively (MPN procedure). Nine parts (1:10 dilution) of sterile distilled water preheated at 45 °C were added, shaked gently and incubated at 36 °C for 24 h. Each suspension (10 mL) was added to 90 mL of Enterobacteriaceae enrichment broth and incubated at 36 °C for 24 h. The duplicate VRBG (violet red bile glucose) agar plates were inoculated with each enrichment culture by streaking a 3 mm loopful to the plate for isolation of single colonies. Petri dishes were incubated at 36 °C for 24 h. Five presumptive *C. sakazakii* colonies from the above plates were picked and each isolate was subcultured by streaking onto a single TSA plate and then incubated at 25 °C for 48–72 h. Yellow pigmented colonies were selected from the TSA plates and confirmed using the API 20E biochemical identification kits according to the manufacturer's instructions (Biomerieux, France). The oxidase test was included. Finally, MPN of *C. sakazakii* was calculated as cells/g of sample based on the number of "tubes" at each dilution in which the presence of *C. sakazakii* was confirmed.

2.4. Determination of microbiological profile of samples

The microbiological profile of *C. sakazakii* positive samples was examined using the methods explained below:

2.4.1. Determination of Enterobacteriaceae

For the determination and enumeration of Enterobacteriaceae, the ISO (2004a) method was used. Sample (25 g) was added to a sterile bag containing 225 mL peptone water and homogenized in a stomacher (Seward) for 2 min. The sample was then serially diluted and 1 mL dilutions was plated on VRBGA (CM1082) using the pour plate method in duplicate. The plates were overlaid with 5 mL of VRBD and incubated for 24 h at 37 °C. Red colonies were counted and oxidase test was applied for biochemical confirmation. Oxidase negative colonies were calculated.

2.4.2. Determination of yeasts and moulds

For yeasts and moulds, counts in products with water activity less than or equal to 0.95, determination was done according to the

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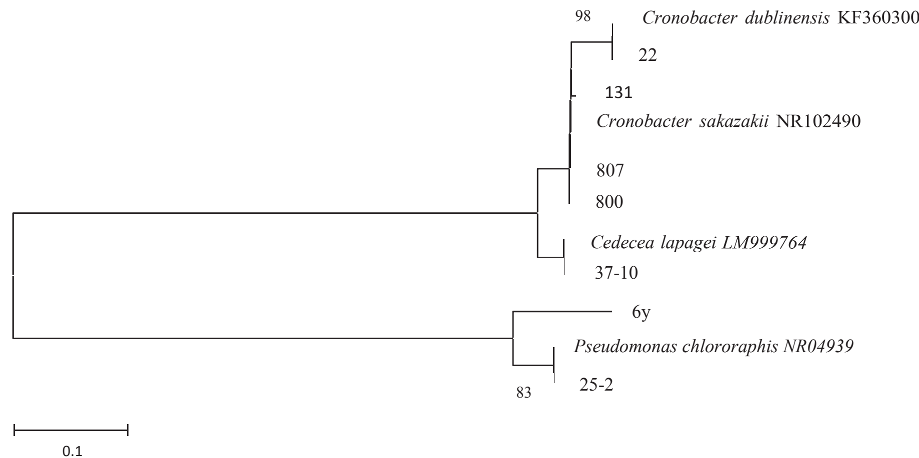

Fig. 1. The dendogram constructed (Neighbor-joining) from the partial 16S sequences of the isolates and the matching sequences from reference strains found in databases (NCBI gene bank).

standard (ISO, 2008). The sample was prepared as it was explained in Section 2.4.2. Sample (0.1 mL) was plated onto DG18 Agar (CM1150 + SR0078 or CM1151) using the surface plate method in duplicate. Plates were incubated for 5–7 days at 25 °C.

2.4.3. Determination of mesophilic aerobic bacteria

For the mesophilic aerobic bacteria, plate count agar (PCA) was used according to the standard (ISO, 2003). Twenty-five g of sample was homogenized in 225 mL peptone water in a stomacher for 2 min. The sample was then serially diluted and 1 mL dilutions were plated on PCA using the pour plate method in duplicate. The plates were incubated at 30 °C for 3 days.

2.4.4. Determination of coliform and Escherichia coli

For the enumeration of coliform and *E. coli* MPN method was used according to the standard ISO (2006) and ISO (2005), respectively. Sample (25 g) was homogenized in 225 mL peptone water in a stomacher for 2 min. The sample was then serially diluted and 1 mL of dilutions was added to each of three tubes of 10 mL of Lauryl Sulphate Tryptose broth (CM0451). Further dilutions were proceed in the same way. The tubes were incubated for 24 h at 37 °C. If negative, tubes were incubated for a further 24 h. For confirmation a loopful of sample/dilutions from each positive tube (gas and/or turbidity) was used to inoculate 10 mL of Brilliant Green Lactose Bile Broth (CM0031). Broths were incubated for 24 h at 37 °C. If negative, tubes were incubated for further 24 h. Positive tubes (gas and/or turbidity) were used to calculate MPN.

2.4.5. Determination of Staphylococcus aureus

For the enumeration of *S. aureus* (coagulase-positive) the Baird Parker (BP) Medium (CM1127 + SR0054) was used (ISO, 1999). 1 mL of serially diluted sample (25 g in 225 mL peptone water) was surface plated onto 3 different BP medium and the plates were incubated at 37 °C for 2 days. The position of typical colonies was marked and transferred into Brain Heart Infusion Broth (CM1135) for confirmation. After incubated at 36 °C for 18–24 h, 0.1 mL was added to Rabbit Plasma (R21050). Precipitations around typical colonies were observed after incubated at 36 °C. If negative, plates were incubated for a further 24 h.

2.4.6. Determination of Listeria monocytogenes

For the determination of *L. monocytogenes*, the ISO (1996) and ISO (2004b) methods were used. For the primary enrichment, 25 g of sample was added into 225 mL Half Fraser Broth (CM0895 + SR0166) and incubated at 30 °C for 24 h. Ottoviani and Agosti (ALOA, CM1084 + SR0244, SR0226) agar and Oxford (CM0856 + SR0140) agar were inoculated by a sterile needle and incubated at 37 °C for 24 h. Typical colonies (5 colonies from each medium), blue green with opaque zones around in ALOA agar and small grey colonies with black circle around in Oxford agar were streaked onto TSYEA (Tryptone Soya Yeast Extract Broth, CM0862 + agar) and incubated for 18–24 h at 35 °C-37 °C. For confirmation catalase test, Gram stain and motility test (Tryptone Soya Yeast Extract Broth, CM0862) were applied. For confirmation of *L. monocytogenes* API *Listeria* kits (Biomerieux, France) were used according to the manufacturer's instructions.

2.4.7. Determination of Salmonella

For the determination of *Salmonella* spp., the ISO (2002) method was used. For the primary enrichment, 25 g of sample was added into 225 mL buffered peptone water and incubated for 18 h at 37 °C. For selective enrichment, 0.1 mL of culture was transferred to 10 mL of RVS broth (Rappaport Vassiliadis broth, CM0866) and incubated for 24 h at 41.5 °C. The other 1 mL of culture was transferred to 10 mL of MKTTn broth (Muller-Kauffmann Tetrathionate Novobiocin, CM1048 + SR0181) and incubated for 24 h at 37 °C. Cultures were then streaked onto both XLD (Xylose Lysine Deoxycholate Agar, CM0469) and Brilliant Green Agar (CM0329) and then incubated for 24 h at 37 °C. Typical colonies were transferred to TSA agar by sterile needle and incubated for 18–24 h at 37 °C. For the biochemical confirmation, API 20 E kits (Biomerieux, France) was used according to the manufacturer's instructions.

3. Results

Two different specific methods namely ISO/IDF (2006) and BAX System PCR assay were applied for the determination of *Cronobacter sakazakii* in PIF, FUF as well as in the ingredients used for the preparation of these products. Each presumptive *C. sakazakii* isolate was then subjected to identification by polymerase chain reaction (PCR) with partial 16S rRNA gene sequencing. *C. sakazakii* positive samples, the number of *C. sakazakii* and non *Cronobacter* spp. in each positive sample and the confirmed names of organisms by molecular methods was presented in Table 1.

Similar results were obtained from ISO/IDF (2006) and BAX System PCR assay for the presumptive *C. sakazakii* in the samples.

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Table 1
Cronobacter sakazakii positive samples using two different methods, and the number of *Cronobacter (sakazakii)* spp. in each positive samples and the confirmed names by 16S rRNA.

Type of Samples	Number of Samples	The number of *Cronobacter sakazakii*				Confirmed Bacterial Species
		Positive Samples		Sample No.	In the Samples (Log_{10} MPN/g)	
		ISO 22964/IDF 210	BAX-PCR			
Milk powder	25	3	3	1	1.04	*Cronobacter sakazakii* (800)
				2	1.18	*Cronobacter sakazakii* (807)
				3	0.32	*Pseudomonas chlororaphis* (6y)
Whey powder	15	2	2	4	0.32	*Cronobacter dublinensis* (22)
				5	0.85	*Cronobacter sakazakii* (131)
Cheese	19	ND[a]	ND	–	ND	ND
Powdered infant formula	40	1	1	6	−0.14	*Cedecea lapagei* (37–10)
Follow-up formula	15	1	1	7	−0.13	*Pseudomonas chlororaphis* (25–2)
Total	114	7	7	–	–	–

[a] Not Detected.

Out of 114 samples; 7 (6.14%) were found to be *C. sakazakii* positive by both methods. Three samples out of 25 (12%) milk powder, 2 out of 15 (13.3%) whey powder, 1 out of 40 (2.5%) powdered infant formula (PIF) and 1 out of 15 (6.7%) follow-up formula were contaminated with presumptive *Cronobacter* spp. *Cronobacter* spp. was not found in cheese samples (n = 15). Since there has been progress and rapid changes in the identification of *Cronobacter* spp. the species identified as *C. sakazakii* by these methods were subjected to 16S rRNA by PCR for the confirmation. Thus, all presumptive *C. sakazakii* strains were checked by the 16S rRNA analysis and the results was given in Fig. 1. Among seven presumptive *C. sakazakii* strains, only three were confirmed by 16S rRNA gene sequencing. The rest of the strains were identified as follows; *Cedecea lapagei* (37–10), *Cronobacter dublinensis* (22) and *Pseudomonas chlororaphis* (6y and 25–2). Based on the results obtained from molecular methods, *C. sakazakii* was not found in infant formula and follow-up formula but in the ingredients (milk powder and whey powder). Although the incidence (1 out of 40) was low, powdered infant formula was found to be contaminated with another opportunistic pathogen *Cedecea lapagei* (37–10).

The incidence of *C. sakazakii* was 8 and 6.7% in milk and whey powders, respectively. The number of *C. sakazakii* was 7–15 MPN/g in milk powders and whey powders. The incidence of *Cedecea lapagei* was 2.5% and the number was less than 10 MPN/g in PIF. The number of *C. sakazakii* should be 0 in 25 g of sample according to the Turkish Codex. *C. dublinensis* was considered as non-pathogenic and *Cedecea lapagei* was not mentioned in the Codex. Only three samples did not meet the limits in the Turkish Codex. *Cedecea lapagei* is also an opportunistic pathogen and should not be found in PIF. To our best knowledge, this is the first study reporting *Cedecea lapagei* in PIF.

The microbiological profile of *Cronobacter* spp. and non *Cronobacter* spp. positive samples were given in Table 2. The number of mesophilic aerobic bacteria (MAB) in samples was between 1.71 log_{10} CFU/g and 4.99 log_{10} CFU/g. Pathogens such as *Salmonella* spp., *Listeria monocytogenes*, and *E. coli* were not detected in any of the samples. Samples were also found free of mould and yeast except sample no. 4 (whey powder). However, coagulase positive *Staphylococcus aureus* was found in all milk powders and a whey powder tested. One milk powder was also positive for coliforms (Table 2). The number of MAB was 1.71 log_{10} CFU/g in infant formula and the number of *Cedecea lapagei* in this sample was 0.72 MPN/g (−0.14 Log_{10} MPN/g; Tables 1 and 2).

4. Discussion

A variety of methods including culture-depended methods, molecular methods, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), pulsed-field gel electrophoresis (PFGE), probe-magnetic separation PCR assay, and ribotype analysis are examples for development of specific analysis of *Cronobacter* spp. in PIF (ISO/IDF, 2006; Lu, Chen, Lu, Lv, & Man, 2014; Mullane et al., 2008; Xu et al., 2014; Zimmermann et al., 2014). The real time PCR-based detection system was developed by Zimmermann et al. (2014), recently. This method includes 12 h of enrichment step and allows 1.00 log_{10} CFU/g of specific detection at the genus level in 24 h. Another rapid method is the probe-magnetic separation PCR assay specific for *Cronobacter* spp. (Xu

Table 2
The microbiological profile of *C. sakazakii* positive samples.

Parameters	The number of microorganisms in the samples						
	Milk Powder			Whey Powder		Powdered Infant Formula	Follow-up formula
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
Mesophilic Aerobic Bacteria (Log_{10} CFU/g)	3.54	4.99	4.04	4.86	4.08	1.71	1.95
Mean[b] *± standard deviation*	*4.19 ± 0.73*			*4.47 ± 0.55*			
Coliforms (Log_{10} MPN/g)	2.04	<0.48	<0.48	<0.48	<0.48	<0.48	<0.48
Escherichia coli (Log_{10} MPN/g)	<0.48	<0.48	<0.48	<0.48	<0.48	<0.48	<0.48
Staphylococcus aureus (Log_{10} CFU/g)	2.72	2.41	1.9	3.18	<1.00	<1.00	<1.00
Mean[b] *± standard deviation*	*2.34 ± 0.41*						
Moulds (Log_{10} CFU/g)	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
Yeasts (Log_{10} CFU/g)	<1.00	<1.00	<1.00	<1.00	2.95	<1.00	<1.00
Listeria monocytogenes (in 25 g)	ND[a]	ND	ND	ND	ND	ND	ND
Salmonella spp. (in 25 g)	ND	ND	ND	ND	ND	ND	ND

[a] Not Detected.
[b] Mean population densities of bacteria isolated from different products were not significantly ($P < 0.05$) different.

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et al., 2014). Although this combination of PMS-PCR is very fast and takes less than 4 h, the limit of detection is 3.00 $\log_{10}$ CFU/g and thus not as low as ISO/IDF (2006). MALDI-TOF MS may be another rapid method of choice. Although, analysis by MALDI-TOF MS alone was more accurate for identification of *Cronobacter* spp. compared with ribotype analysis, MALDI-TOF MS typing and ribotype analysis gave more discrimination between strains (Lu et al., 2014). In this study, two different methods were applied for the specific detection of *C. sakazakii* in PIF, FUF and dairy based ingredients. ISO/IDF (2006) method is a culture based method and includes pre-enrichment, selective enrichment, and isolation of presumptive *C. sakazakii* colonies and finally identification of yellow colonies in TSA agar as *C. sakazakii*. Although this method takes six to seven days to obtain a positive result, it is easy to use, additional technical assistance is not needed, expensive equipment is not necessary and finally, it is accepted as a gold standard. BAX® System PCR assay designed for *C. sakazakii* was used as a rapid method. This PCR-based detection system includes 23–25 h of enrichment and allows specific detection in 4 h. Seven out of 114 samples were positive for presumptive *C. sakazakii* in this study. Since there has been progress and rapid changes in the identification of *Cronobacter* spp., the species identified as *C. sakazakii* were subjected to 16S rRNA gene sequencing for confirmation. Among seven strains, four strains were not confirmed and were identified as *C. dublinensis*, *Cedecea lapagei* and *Pseudomonas chlororaphis* by 16S rRNA gene sequencing. Among the positive samples, *C. dublinensis* was considered as non-pathogenic and *Cedecea lapagei* was not mentioned in the national and the international standards; only three samples did not meet the limits.

In this study, the prevalence of *C. sakazakii* was 7.5% in the ingredients of PIF such as milk powder and whey powder and the number of *C. sakazakii* in the samples were between 7 and 15 MPN/g. *Cronobacter sakazakii* were isolated from PIF (Pan et al., 2014) and follow-up formula (Chap et al., 2009; Iversen, Lane, & Forsythe, 2004) in a variety of studies. The rate of contaminated ingredients of PIF (7.5%) was lower in this study than the rates (12.8%) in other studies (Pan et al., 2014).

C. sakazakii was the only *Cronobacter* species isolated from follow-up foods and infant foods in the study organized by seven countries (Chap et al., 2009). *C. sakazakii* was isolated from 1/136 (0.7%) follow-up formulas, and 22/179 (12%) infant foods. For the follow-up formulas the intended age is > 6 months and consumed as liquid as described by CAC (2009) whereas for infant formulas were intended for infants aged 6–12 months. In Turkey, all foods with intended infant age >6 was considered as follow-up formula. The result in this study (2.5% *Cedecea lapagei*) was lower than the result obtained from the collaborative study (Chap et al., 2009) mentioned above (7.3% *C. sakazakii*) and lower than the result of the studies by Iversen et al. (2004), Pan et al. (2014); and Yao et al. (2012) which were 10, 12.8 and 13% respectively.

Cronobacter sakazakii and related species were also isolated from ingredients of the baby foods. Milk and whey powder are two important ingredients for manufacturing PIF and follow-up formula, whereas cheese (feta cheese) has been used as an ingredient in the follow-up formula. These three ingredients are used for the production of various food products for children and adults as well. *C. sakazakii* (Lehner & Stephean, 2004), *C. malonaticus* and *C. dublinensis* were previously isolated from milk powder (Turcovsky, Kunikova, Drahovska, & Kachkova, 2011). In a survey, *C. sakazakii* was detected in milk powder (5%) and feta cheese (4%) but not in whey powder in Turkey (Gokmen, Tekinsen, & Gurbuz, 2010). In this study, out of 25 milk powder, three (12%) were contaminated with *C. sakazakii* and out of 15 whey powder, one (6.6%) was contaminated with *C. sakazakii*. No *Cronobacter* spp. was detected in feta cheese.

The microbiological profile of contaminated samples was also determined in this study. The number of mesophilic aerobic plate count was between 1.7 (powdered infant formula) and 4.99 (milk powder) $\log_{10}$ CFU/g in the samples. Pathogens such as *Salmonella* spp, *Listeria monocytogenes*, and *E. coli* and moulds were not detected in any of the samples. However, coagulase positive *Staphylococcus aureus* was found in all milk powder and a whey powder tested. A milk powder was also positive for coliforms (Table 2).

The number of MAB was 1.71 and 1.95 $\log_{10}$ CFU/g in infant formula and follow-up formula (Table 2), respectively. Only 1 out of 40 PIF was positive and the species isolated from this sample was reidentified as *Cedecea lapagei* (37–10). The number of *Cedecea* in this sample was less than one.

In a coordinated survey for *Cronobacter* spp. in powdered infant formula and follow-up formula in seven countries, MAB ranged from <2 to >5 $\log_{10}$ CFU/g (Chap et al., 2009). Some samples had high MAB but not contaminated with *Cronobacter* whereas the others had low MAB but contaminated with *C. sakazaii*. Since follow-up formula is only part of the infant's diet, it was suggested to include *Salmonella* spp. and not *Cronobacter* spp. for establishing microbiological criteria for follow-up formula.

5. Conclusion

Cronobacter sakazakii was not found in PIF and FUF but in the ingredients used for the preparation of PIF and FUF in this study. However, *Cedecea lapagei* was detected for the first time in PIF. Since the pathogens such as coliforms, *E. coli*, *L. monocytogenes* and *Salmonella* were not detected in any of the PIF and FUF samples and the MAB count is low, it is thought that the products were not manufactured under poor hygienic conditions. Although *Cronobacter sakazakii* was not detected in cheese, this does not indicate that milk as well as other ingredients used in cheese manufacturing or the environment was free of this pathogen. Since the cheese is a fermented product, *Cronobacter* and related species could be affected by the background flora. *Cronobacter sakazakii* and *Cedecea lapagei* are opportunistic pathogens, they may increase under suitable conditions and cause health hazards. Since contamination during processing and after pasteurization might be possible, effective control measures and systems such as HACCP and Good Manufacturing Practices should be applied to reduce the contamination of *Cronobacter* spp. and related species.

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