Prevalence and subtyping of *Cronobacter* species in goat milk powder factories in Shaanxi province, China

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ABSTRACT

Cronobacter spp. are opportunistic pathogens that can cause serious diseases in neonates and infants via consumption of contaminated milk powder. To determine *Cronobacter* spp. contamination status, 632 samples, including 15 evaporated milk, 45 intermediate powder, 150 finished products, and 422 manufacturing environment samples, were collected from 3 goat milk powder factories in Shaanxi province, China, from July 2013 to April 2014. The recovered *Cronobacter* isolates were subtyped using pulsed-field gel electrophoresis to trace the potential dissemination routes during the whole production processing. Sixty-seven *Cronobacter* spp. isolates were recovered. The prevalence rates in manufacturing environment, intermediate powder, and finished products were 92.5, 6.0, and 1.5%, respectively. The predominant species were *Cronobacter sakazakii* (88.1%); no *Cronobacter turicensis*, *Cronobacter condimenti*, or *Cronobacter dublinensis* were detected. Sixty-seven *Cronobacter* isolates were grouped in 26 clusters by pulsed-field gel electrophoresis, and substantial genetic similarity was observed among isolates from different sampling sites in the same factory. Isolates in the main clusters were commonly recovered from intermediate powder, floor powder, and shoes. These data indicated that air, powder, and personnel movement were potential routes for *Cronobacter* dissemination, and manufacturing environment is the key control point for *Cronobacter* contamination.

Key words: *Cronobacter* spp., goat milk powder, pulsed-field gel electrophoresis

INTRODUCTION

Enterobacter sakazakii is a gram-negative and facultative anaerobic organism that was originally named “yellow-pigmented *Enterobacter cloacae*,” until 1980 when it was known as *E. sakazakii* (Farmer et al., 1980). Recent studies indicated that *E. sakazakii* was a novel genus and renamed as *Cronobacter* spp., including 7 species: *Cronobacter sakazakii*, *Cronobacter malonaticus*, *Cronobacter muytjensii*, *Cronobacter dublinensis*, *Cronobacter turicensis*, *Cronobacter condimenti*, and *Cronobacter universalis* (formerly *Cronobacter* genomospecies 1; Joseph et al., 2012). Isolates in *Cronobacter* spp. have been found to carry different virulence factors and are considered to be opportunistic pathogens that can cause meningitis, necrotizing enterocolitis, and bacteremia in premature and immunocompromised infants (Caubilla-Barron et al., 2007; Mullane et al., 2007; Townsend et al., 2008; Craven et al., 2010), with fatality rates ranging from 40 to 80% (Bowen and Braden, 2006; Yan et al., 2012). Although there are 7 species of *Cronobacter*, only isolates of *C. sakazakii*, *C. malonaticus*, and *C. turicensis* are associated with neonatal infections (Kucerova et al., 2010).

Enterobacter sakazakii has been isolated from milk powder (Farmer et al., 1980; Iversen et al., 2004). The composition of dry foods and infant formula combined with their low water activity ($a_w \sim 0.2$) significantly affected the survival of *Cronobacter* spp. in these foods (Breeuwer et al., 2003; Ziad et al., 2009). The occurrence of *E. sakazakii* in milk powder production environment has been documented (Cox et al., 1988); *E. sakazakii* can gain access to the powder from the environment or from the addition of ingredients at the powder stage (Nazarowec-White and Farber, 1997). *Cronobacter* spp. have been isolated from a wide range of habitats, which include milk powder and formula constituents, and from environments within manufacturing plants (Reyad et al., 2007), as well as from animal food sources such as milk, meat, and fish, or products made from these foods

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(Miriam, 2007). Over recent decades, cases involving outbreaks and sporadic *Cronobacter* spp. infection in infants are under-reported except in France (Caubilla-Barron et al., 2007), the United States (Himelright et al., 2002; Mary et al., 2014), and in some Asian countries, such as China (Xu et al., 2014) and India (Ray et al., 2007). Although not all cases were associated with *Cronobacter* spp., contaminated powdered infant formula studies have shown that contamination of powdered infant formula is commonly involved in infections in infants (Himelright et al., 2002; Mullane et al., 2007; El-Sharoud et al., 2009).

Cronobacter spp. can grow in infant formula milk at temperatures ranging from 6 to 45°C (Iversen et al., 2004). Although they are heat sensitive, they can tolerate high desiccation and osmotic stress and survive during milk powder processing (Breeuwer et al., 2003; Arku et al., 2008). Moreover, the formation of biofilm of *Cronobacter* spp. helps the organisms survive in milk powder and on the surface of processing utilities (Iversen et al., 2004; Hartmann et al., 2010; Du et al., 2012) and enables the organisms to resist multiple stress conditions, including water, nutrient shortages, and presence of biocides (Iversen et al., 2004; Kives et al., 2006).

Goat milk accounts for about 2.3% of the worldwide milk production (Claeys et al., 2014). Goat milk-based infant formula is becoming increasingly preferred by consumers (Xi et al., 2015) because of its specific composition and high nutritional utilization (Alferez et al., 2001; Haenlein, 2004; Mwenze and Kiplagat, 2011). China is the largest goat breeding and production country in the world (Luo, 2009; Thiruvankadan, 2012), and Shaanxi province is one of the major living areas for goats in China (Luo, 2009). Goat milk powder

is a characteristic material of infant formula in Shaanxi province. The microbial pollution of goat milk powder deserves to be researched. However, contamination of *Cronobacter* spp. in milk powder-processing factories has been rarely documented in China, especially in those processing goat milk powder.

In our study, 3 goat milk powder-processing factories were sampled to assess the prevalence of *Cronobacter* over a 10-mo period, and isolates were subtyped by pulsed-field gel electrophoresis (PFGE) to identify the potential dissemination routes of *Cronobacter* spp. and to develop effective control procedures to reduce the risk of *Cronobacter* spp. contamination in goat milk powder.

MATERIALS AND METHODS

Sampling Plan

The majority of the stages of goat milk powder processing and manufacturing environment of 3 goat milk powder factories (referred to as A, B, and C) were selected for samples collection between July 2013 and April 2014. The detailed sampling information was listed in Table 1. The intervals between each sampling were 8 wk; in total, 632 samples were collected.

Air samples were collected by using *Cronobacter* spp. chromogenic plates (*E. sakazakii* Chromogenic Medium; Beijing Land Bridge Technology Ltd., Beijing, China), which were exposed in the air in air filtration rooms, spray drying rooms, fluidized bed rooms, and packaging rooms for 30 min. Fluid samples (50 mL) included water collected from the drainage system and evaporated milk. Powder samples included finished powder products (100 g), intermediate powder (100 g),

Table 1. Locations and sample numbers in 3 goat milk powder factories

Sample type	Location	A	B	C	Total
Air samples	Air filtration rooms	NT ¹	1	1	2
	Spray drying rooms	1	1	1	3
	Fluidized bed rooms	1	1	NT	2
	Packaging rooms	1	1	1	3
Fluid	Drain	NT	1	1	2
	Evaporated milk	1	1	1	3
	Spray drying rooms	NT	1	1	2
Powder	Floor powder of fluidized bed rooms	1	1	NT	2
	Intermediate powder	1	1	1	3
	Floor powder of packaging rooms	1	1	1	3
	Final products	1	1	1	3
	Soil around workshop	1	1	1	3
	Air filtration rooms	3	2	2	7
	Spray drying rooms	5	4	4	13
Swabs	Fluidized bed rooms	2	1	2	5
	Packaging rooms	6	6	7	19
	Total locations	25	25	25	75

¹Not tested.

floor powder (50 g) in spray drying rooms, fluidized bed rooms, and packaging rooms, and soil around the workshop (100 g).

Sites for swab sampling were (1) air filtration rooms, including air filter, wall, and floor; (2) spray drying rooms, including wall, floor, outside dryer, inside dryer, entrance to dryer, and dryer platform; (3) fluidized bed rooms, including wall, floor, and inside and outside of the fluidized beds; and (4) packaging rooms, including shoes, hands, wall, floor, transmission belts, entrances to packaging areas, powder box, bag-filling platform, and associated processing equipment. All swab samples were collected directly over a 25-cm² surface area with sterile cotton swabs moistened in sterilized water.

Isolation and Identification of *Cronobacter* spp.

Samples were tested for the presence of *Cronobacter* spp. according to the standard procedures described in the National Health and Family Planning Commission of the People's Republic of China (2010; GB 4789.40–2010).

For air samples, chromogenic medium (*E. sakazakii* Chromogenic Medium; Beijing Land Bridge Technology Ltd.) exposed to air was incubated at 42°C for 24 h; then, typical blue-green colonies were picked out and grown overnight at 37°C on tryptone soy agar (Beijing Land Bridge Technology Ltd.), and identified using API 20E diagnostic strips (bioMérieux Company, La Balme, France).

For liquid and powder samples and swabs, each sample and swab were put into 100 mL of buffered peptone water (Difco, Sparks, MD) and incubated at 37°C for 24 h. Then, 1 mL of the culture buffered peptone water was transferred to 10 mL of modified lauryl sulfate tryptone (Beijing Land Bridge Technology Ltd.)-vancomycin (10 µg/mL of vancomycin; Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China) broth, and incubated at 42°C for 24 h. A loopful of modified lauryl sulfate tryptone-vancomycin broth was streaked onto chromogenic medium agar and incubated at 42°C for 24 h. The typical blue-green colonies were picked and re-streaked on tryptone soy agar and plates were incubated at 37°C overnight and identified by API 20E diagnostic strips. All *Cronobacter* spp. isolates were identified to species level by PCR using different primer pairs based on *rpoB* sequences as previously described (Stoop et al., 2009; Lehner et al., 2012).

Determination of Molecular Subtypes by PFGE

All *Cronobacter* spp. isolates recovered from the goat milk powder factories were subtyped by PFGE that was performed according to standardization of

PFGE protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet (Ribot et al., 2006). *Salmonella* Braenderup H9812 was used as the control strain. Briefly, a Dade Microscan Turbidity Meter (Dade Behring, Deerfield, IL) was used to adjust the optical density value of fresh *Cronobacter* spp. cell suspensions to 0.48 to 0.52. Agarose plugs were prepared and lysed in a 54°C water bath for 2 h in 5 mL of cell lysis buffer [50 mM Tris-HCl (pH 8.0), Sigma Chemical Co. St. Louis, MO; 50 mM EDTA (pH 8.0), Sigma Chemical Co.; 1% Sarcosyl, Sigma Chemical Co., 0.1 mg/mL of proteinase K (TaKaRa, Dalian, China)] with constant agitation (150–175 rpm). Agarose-embedded DNA were digested by 50 U of *Xba*I (TaKaRa) for 1.5 to 2 h in a water bath at 37°C. The DNA fragments were separated by electrophoresis in 1% agarose gel (Seakem Gold, Rockland, ME) in 0.5 × Tris boric acid buffer at 14°C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA) with pulse times of 2.16 to 63.8 s. Gels were stained with ethidium bromide and DNA bands were visualized under UV transillumination (Bio-Rad).

The DNA fingerprints were stored as tagged image file format (TIFF) files and dendrograms were created using the BioNumerics Software (Applied-Maths, Kortrijk, Belgium) using the DICE coefficient and the unweighted pair group method with arithmetic means (UPGMA) with an optimization value of 0.5 and a 1.0% band position tolerance. The genetic diversity of the *Cronobacter* spp. isolates was compared at a level of similarity of 90%.

RESULTS

Prevalence of *Cronobacter* spp.

Among 632 samples, 67 (10.6%) were positive for *Cronobacter* spp., the prevalence rates of samples in factories A, B, and C were 15.1, 13.4, and 2.1%, respectively. Twenty-six out of 75 sampling sites were detected *Cronobacter*-positive (Table 2). As shown in Table 3, the majority (92.5%) of *Cronobacter* isolates were recovered from the manufacturing environment settings. 6.0% of the intermediate powder, 1.5% of the finished products, and 9.0% of the samples collected in air filtration rooms were detected positive for *Cronobacter* spp. respectively. No *Cronobacter* spp. isolate was detected in air samples collected in spray drying rooms. The highest rate (41.8%) was found in samples collected in spray drying rooms. In fluidized bed rooms, *Cronobacter* spp. isolates were commonly found in floor powder (6.0%), fluidized bed (4.5%), air (1.5%), and fluidized bed floor (1.5%). In packaging rooms, *Cronobacter* spp. isolates were prevalent in packaging platform

Table 2. Distribution of *Cronobacter* spp. in 3 goat milk powder factories

Factory	No. of samples	No. of positive samples (%)	No. of sampling points	No. of positive sampling points (%)
A	205	31 (15.1)	25	14 (56.0)
B	238	32 (13.4)	25	10 (40.0)
C	189	4 (2.1)	25	2 (8.0)
Total	632	67 (10.6)	75	26 (34.7)

(3.0%), shoes (3.0%), floor powder (1.5%), transmission belt (1.5%), powder box (1.5%), and packaging floor (1.5%; Figure 1). *Cronobacter* isolates were detected from drain (3.0%) and soil (6.0%) as well.

PCR Identification of Species

The *RpoB* amplification results revealed that *C. sakazakii* (n = 59/67, 88.1%) was the predominant species of *Cronobacter* isolates, followed by *C. malonaticus* (n = 6/67, 9.0%), *C. muytjensii* (n = 1/67, 1.5%), and *C. universalis* (n = 1/67, 1.5%; Table 4). No isolates of *C. turicensis*, *C. condimenti*, and *C. dublinensis* were detected. The distribution of *C. sakazakii* (n = 59) in factories A, B, and C was 30 (44.8%), 25 (37.3%), and 4 (6.0%) isolates, respectively. All *C. muytjensii* isolates were detected in factory A, and all *C. malonaticus* and *C. universalis* isolates were detected in factory B.

PFGE Analysis

Sixty-seven *Cronobacter* spp. isolates were categorized into 26 different PFGE clusters (Figure 1). Forty-five of 67 isolates were grouped into 5 main clusters

(named S-1 to S-5). In each cluster, most of the isolates were recovered from the same factory.

Isolates belonging to S-1 and S-4 were all recovered from samples collected in factory A. Isolates in cluster S-1 (23.9%) represented 8 different sampling points including intermediate powder and other various environmental settings. Isolates recovered from samples in prepackaged product, shoes, floor powder of fluidized bed rooms, and packaging rooms could be found in cluster S-1 and isolates recovered from shoes and powder box in packaging rooms could be found in cluster S-4, which indicated that the contaminated shoes and floor powder could be the main contamination source for *Cronobacter* spp. in factory A. Similar to factory A, isolates belonging to S-2, S-3, and S-5 were mainly recovered from factory B. Isolates in cluster S-2 were commonly recovered from 8 different sampling sites including air, spray drying, entrances to spray drying areas, inside spray drying and spray drying platform, packaging platform, drain, and intermediate powder. Isolates in cluster S-3 and S-5 were recovered from spray drying areas including spray drying, entrances to spray drying, spray drying platform, and floor powder, which indicated that spray drying areas were principal *Cronobacter* spp. contamination source in factory B.

Table 3. Distribution of *Cronobacter* spp.-positive samples in different sample types of 3 goat milk powder factories

Sample type	Location	Positive locations, ¹ % (n positive/n tested, prevalence %)	Distribution of positive isolates, ² % (n positive/n tested, prevalence %)
Air samples	Air filtration rooms	3.8 (1/2, 50.0)	4.5 (3/10, 30.0)
	Spray drying rooms	0.0 (0/3, 0.0)	0.0 (0/15, 0.0)
	Fluidized bed rooms	3.8 (1/2, 50.0)	1.5 (1/10, 10.0)
	Packaging rooms	0.0 (0/3, 0.0)	0.0 (0/15, 0.0)
Fluid	Drain	3.8 (1/2, 50.0)	3.0 (2/10, 20.0)
	Evaporated milk	0.0 (0/3, 0.0)	0.0 (0/15, 0.0)
Powder	Floor powder of spray drying rooms	3.8 (1/2, 50.0)	7.5 (5/14, 35.7)
	Floor powder of fluidized bed rooms	3.8 (1/2, 50.0)	6.0 (4/11, 36.4)
	Floor powder of packaging rooms	3.8 (1/3, 033.3)	1.5 (1/8, 12.5)
	Intermediate powder	7.7 (2/3, 66.7)	6.0 (4/45, 8.9)
	Final products	3.8 (1/3, 33.3)	1.5 (1/150, 0.67)
	Soil around workshop	7.7 (2/3, 66.7)	6.0 (4/21, 19.0)
Swabs	Air filtration rooms	3.8 (1/7, 14.3)	4.5 (3/49, 6.1)
	Spray drying rooms	26.9 (7/13, 53.8)	41.8 (28/91, 30.8)
	Fluidized bed rooms	7.7 (2/5, 40.0)	6.0 (4/35, 11.4)
	Packaging rooms	19.2 (5/19, 26.3)	10.4 (7/133, 5.3)

¹The ratio of number of positive point to 26 (total number of positive points).

²The ratio of number of positive samples of each sampling point to 67 (total number of positive samples).

Figure 1. XbaI macrorestriction DNA clusters of Cronobacter isolates from 3 milk powder factories. Legend represents epidemiologic relationships.

Table 4. Species of 67 *Cronobacter* spp. isolates by PCR

Species	Factory	Prevalence, % (n positive/n tested)
<i>Cronobacter sakazakii</i>	A	44.8 (30/67)
	B	37.3 (25/67)
	C	6.0 (4/67)
<i>Cronobacter malonaticus</i>	B	9.0 (6/67)
<i>Cronobacter muytjensii</i>	A	1.5 (1/67)
<i>Cronobacter universalis</i>	B	1.5 (1/67)

DISCUSSION

In our study, prevalence of *Cronobacter* spp. in manufacturing environment, intermediate powder, and finished products of 3 goat milk powder factories was investigated in Shaanxi province, China. The recovered isolates were subtyped using PFGE to determine their clonal relatedness and assess their potential dissemination routes. The overall prevalence (10.6%) of *Cronobacter* spp. isolates was consistent with the previous studies in the Netherlands (Kandhai et al., 2004) and Ireland (Mullane et al., 2007), but different from the findings in Australia (Townsend et al., 2008). The variation in prevalence could be attributed to differences in regions, sampling plan, sample types, and so on. Our results showed that different contamination rates exist in different goat milk powder factories.

Cronobacter spp.-positive samples could be found in various manufacturing environment in 3 factories, which is in agreement with previous results (Mullane et al., 2007; Craven et al., 2010). In factory A, *Cronobacter* isolates on the floor of air filtration rooms might contaminate the whole air filtration system and result in cross-contamination during the goat milk powder processing, which indicated that air filters should be replaced or cleaned and disinfected regularly to maintain hygiene. *Cronobacter* spp. contamination also occurred in spray drying rooms, where the workers were frequently exposed to them; thus, personnel movement was a potential route for *Cronobacter* transmission. Cluster S-1 and the cluster next to it contained 18 isolates, which were isolated in fluidized bed rooms (air, floor, floor powder, and fluidized bed) and packaging rooms (shoes, transmission belt, powder box, floor, floor powder). This suggested that air and powder movement were also important potential routes for *Cronobacter* transmission. Regardless of the routes of spread and specific circumstance of each plant, *Cronobacter* contamination would be reduced after installing air filtration systems to prevent unfiltered air entering into hygienic areas, especially areas where air could contact the products directly. Proper disposal of floor

was another important measure to reduce cross-contamination. Therefore dedicated channels for floor powder and other waste should be set up in these plants. In packaging rooms, 2 isolates were detected from shoes, which indicated that the cleaning measures must be taken in the entrances to prevent the contamination as well. For factory B, 2 sampling points, including drainage and packaging platform, were noteworthy. As the water in pipes was used to clean the infrastructure and equipment, presence of *Cronobacter* isolates might result that all the pipes in the factory were contaminated. Moreover, *Cronobacter* spp. have the ability to form biofilm, which can help the organisms to attach to surface, colonize, and resist multiple stress conditions (Iversen et al., 2004; Kives et al., 2006). Drain systems are a key area to decrease *Cronobacter* spp. prevalence if a hazard analysis critical control point system is employed in the factory. The packaging platform surface is located in a highly hygienic area and required to be cleaned by dry cleaning frequently (FAO/WHO, 2006). Compared with factories A and B, factory C had the lowest *Cronobacter* spp. prevalence and maintained highly hygienic conditions. In this factory, filtration systems were used in highly hygienic areas, and these systems were regularly cleaned and disinfected. In addition, floor powder was stored in airtight containers and dry cleaning was applied to avoid the increasing risk of contamination by wet cleaning, which can cause water residue to contribute to multiplication of microorganisms. All of these measures indicated that strict cleaning programs in manufacturing environment can effectively reduce the organisms.

Six percent of the intermediate powder and 1.5% of the finished products were *Cronobacter* spp.-positive. These rates were lower than the rate in Austria (Hein et al., 2009). To reduce the presence of *Cronobacter* spp. in the finished products, the key step is to reduce their levels in the manufacturing environment and to avoid their entry into highly hygienic areas. The effective control measures are based on strict adherence to good manufacturing practices and hazard analysis critical control point principles depending on the characteristics of the microorganisms.

In our study, the majority of the *Cronobacter* isolates were *C. sakazakii*, which is consistent with the previous study in Canada, the European Union, and China (Müller et al., 2013; Sonbol et al., 2013; Xu et al., 2014). This finding was consistent with the findings that *C. sakazakii* is the most common species in terms of isolation frequency (Müller et al., 2013). Sonbol et al. (2013) only found *C. sakazakii* and *C. malonaticus*, whereas the other 5 species were not identified, which differed from our study that 4 species were identified.

Only powdered formulas were investigated in Sonbol et al. (2013), whereas samples were surveyed.

The PFGE was performed to assess the clonal relatedness of the *Cronobacter* spp. isolates recovered from various sampling sites of the 3 factories. In our study, all PFGE clusters of the isolates were unique within each factory, and substantial genetic similarity of the isolates was observed among the different sampling locations in the same factory. Some isolates recovered from different sampling sites showed same or similar PFGE genotype, which indicated that *Cronobacter* spp. isolates in the manufacturing environment may be the source for contamination. For example, isolates in a certain cluster exhibited high genetic homogeneity, and these isolates were all detected from processing steps including spray drying, air and floor powder in fluidized bed rooms, transmission belt, floor, shoes, and floor powder in packaging rooms; and from intermediate powder before blending with other nutrients. Additionally, similar PFGE patterns were identified in isolates from manufacturing environment, intermediate powder, and finished product (S-1 to S-5), which suggested that particular *Cronobacter* spp. isolates may exist in the facility and they were possibly responsible for the contamination of the manufacturing environment and powdered product.

Our study is the first to assess the prevalence of *Cronobacter* spp. and potential transmission routes of contamination in goat milk powder factories in Shaanxi province in China. Air, powder, personnel movement, and inappropriate cleaning methods are potential pathways for *Cronobacter* spp. transmission. Moreover, our study indicates that the manufacturing environment is the predominant source for contamination. Substantial genetic similarity was observed among isolates in different sampling sites in the same factory, suggesting that particular bacterial strains existed in the facility. Appropriate hygiene regimens should be taken by industries to avoid the risk of bacterial transmission within environment and the proliferation of the microorganisms. Meanwhile, further understanding of the contamination routes to prevent of *Cronobacter* spp. in goat milk powder factory is necessary, which would be of benefit for the establishment of appropriate policies.

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REFERENCES

- Alfrez, M. J. M., M. Barrionuevo, I. Lopez Aliaga, M. R. Sanz-Sampelayo, F. Lisbona, J. C. Robles, and M. S. Campos. 2001. Digestive utilization of goat and cow milk fat in malabsorption syndrome. *J. Dairy Res.* 68:451–461.
- Arku, B., N. Mullane, E. Fox, S. Fanning, and K. Jordan. 2008. *Enterobacter sakazakii* survives spray drying. *Int. J. Dairy Technol.* 61:102–108.
- Bowen, A. B., and C. R. Braden. 2006. Invasive *Enterobacter sakazakii* disease in infants. *Emerg. Infect. Dis.* 12:1185–1189.
- Breeuwer, P., A. Lardeau, M. Peterz, and H. M. Joosten. 2003. Desiccation and heat tolerance of *Enterobacter sakazakii*. *J. Appl. Microbiol.* 95:967–973.
- Caubilla-Barron, J., E. Hurrell, S. Townsend, P. Cheetham, C. Loc-Carrillo, O. Fayet, M.-F. Prre, and S. J. Forsythe. 2007. Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from an outbreak resulting in fatalities in a neonatal intensive care unit in France. *J. Clin. Microbiol.* 45:3979–3985.
- Claeys, W. L., C. Verraes, S. Cardoen, J. De Block, A. Huyghebaert, K. Raes, K. Dewettinck, and L. Herman. 2014. Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Contr.* 42:188–201.
- Cox, L. J., N. Keller, and M. van Schothorst. 1988. The use and misuse of quantitative determinations of *Enterobacteriaceae* in food microbiology. *Soc. Appl. Bacteriol. Symp. Ser.* 17:237S–249S.
- Craven, H. M., C. M. McAuley, L. L. Duffy, and N. Fegan. 2010. Distribution, prevalence and persistence of *Cronobacter (Enterobacter sakazakii)* in the nonprocessing and processing environments of five milk powder factories. *J. Appl. Microbiol.* 109:1044–1052.
- Du, X. J., F. Wang, X. Lu, B. A. Rasco, and S. Wang. 2012. Biochemical and genetic characteristics of *Cronobacter sakazakii* biofilm formation. *Res. Microbiol.* 163:448–456.
- El-Sharoud, W. M., S. O'Brien, C. Negro, C. Iversen, S. Fanning, and B. Healy. 2009. Characterization of *Cronobacter* recovered from dried milk and related products. *BMC Microbiol.* 9:24.
- FAO/WHO. 2006. *Enterobacter sakazakii* and *Salmonella* in powdered infant formula. MRA Series 10. Geneva: World Health Organisation. Accessed Dec. 10, 2013. <http://www.who.int/foodsafety/publications/micro/mra10.pdf>.
- Farmer, J. J., M. A. Asbury, F. W. Hickman, and D. J. Brenner. 1980. *Enterobacter sakazakii*: A new species of “*Enterobacteriaceae*” isolated from clinical specimens. *Int. J. Syst. Bacteriol.* 30:569–584.
- Friedemann, M. 2007. *Enterobacter sakazakii* in food and beverages (other than infant formula and milk powder). *Int. J. Food Microbiol.* 116:1–10.
- Haenlein, G. F. W. 2004. Goat milk in human nutrition. *Small Rumin. Res.* 51:155–163.
- Hartmann, I., P. Carranza, A. Lehner, R. Stephan, L. Eberl, and K. Riedel. 2010. Genes involved in *Cronobacter sakazakii* biofilm formation. *Appl. Environ. Microbiol.* 76:2251–2261.
- Hein, I., B. Gadzov, D. Schoder, H. Foissy, B. Malorny, and M. Wagner. 2009. Temporal and spatial distribution of *Cronobacter* isolates in a milk powder processing plant determined by pulsed-field gel electrophoresis. *Foodborne Pathog. Dis.* 6:225–233.
- Himelright, I., E. Harris, V. Lorch, M. Anderson, T. Jones, A. Craig, M. Kuehnert, T. Forster, M. Arduino, B. Jensen, and D. Jernigan. 2002. *Enterobacter sakazakii* infections associated with the use of powdered infant Formula-Tennessee, 2001. *JAMA* 287:2204–2205.
- Iversen, C., M. Lane, and S. J. Forsythe. 2004. The growth profile, thermotolerance and biofilm formation of *Enterobacter sakazakii* grown in infant formula milk. *Lett. Appl. Microbiol.* 38:378–382.
- Jaradat, Z. W., Q. O. Ababneh, I. M. Saadoun, N. A. Samara, and A. M. Rashdan. 2009. Isolation of *Cronobacter* spp. (formerly *Enterobacter sakazakii*) from infant food, herbs and environmental samples and the subsequent identification and confirmation of the isolates using biochemical, chromogenic assays, PCR and 16S rRNA sequencing. *BMC Microbiol.* 9:225.
- Joseph, S., E. Cetinkaya, H. Drahovska, A. Levican, M. J. Figueras, and S. J. Forsythe. 2012. *Cronobacter condimenti* sp. nov., isolated from spiced meat, and *Cronobacter universalis* sp. nov., a species

- designation for *Cronobacter* sp. genomospecies 1, recovered from a leg infection, water and food ingredients. *Int. J. Syst. Evol. Microbiol.* 62:1277–1283.
- Kandhai, M. C., M. W. Reij, K. Van Puyvelde, O. Guillaume-Gentil, R. R. Beumer, and M. Van Schothorst. 2004. A new protocol for the detection of *Enterobacter sakazakii* applied to environmental samples. *J. Food Prot.* 67:1267–1270.
- Kives, J., B. Orgaz, and C. Sanjose. 2006. Polysaccharide differences between planktonic and biofilm-associated EPS from *Pseudomonas fluorescens* B52. *Colloids Surf. B Biointerfaces* 52:123–127.
- Kucerova, E., S. W. Clifton, X. Q. Xia, F. Long, S. Porwollik, L. Fulton, C. Fronick, P. Minx, K. Kyung, W. Warren, R. Fulton, D. Feng, A. Wollam, N. Shah, V. Bhonagiri, W. E. Nash, K. Halls-worth-Pepin, R. K. Wilson, M. McClelland, and S. J. Forsythe. 2010. Genome sequence of *Cronobacter sakazakii* BAA-894 and comparative genomic hybridization analysis with other *Cronobacter* species. *PLoS ONE* 5:e9556.
- Lehner, A., C. Fricker-Feer, and R. Stephan. 2012. Identification of the recently described *Cronobacter condimenti* by an *rpoB*-gene-based PCR system. *J. Med. Microbiol.* 61:1034–1035.
- Luo, J. 2009. Dairy goat production in China. Tilahun Sahl, director, E (Kika) de la Garza, American Institute for Goat Research, Langston University, Langston, OK.
- Mullane, N. R., P. Whyte, P. G. Wall, T. Quinn, and S. Fanning. 2007. Application of pulsed-field gel electrophoresis to characterise and trace the prevalence of *Enterobacter sakazakii* in an infant formula processing facility. *Int. J. Food Microbiol.* 116:73–81.
- Müller, A., R. Stephan, C. Fricker-Feer, and A. Lehner. 2013. Genetic diversity of *Cronobacter sakazakii* isolates collected from a Swiss infant formula production facility. *J. Food Prot.* 76:883–887.
- Mwenge, P. M., and I. C. Kiplagat. 2011. Functional Properties of Goat Milk: A Review Paper. Animal Production Society of Kenya, Nairobi.
- National Health and Family Planning Commission of the People's Republic of China. 2010. National food safety standard Food microbiological examination: *Enterobacter sakazakii*, GB 4789.40–2010. National Health and Family Planning Commission of the People's Republic of China.
- Nazarowec-White, M., and J. M. Farber. 1997. *Enterobacter sakazakii*: A review. *Int. J. Food Microbiol.* 34:103–113.
- Patrick, M. E., B. E. Mahon, S. A. Greene, J. Rounds, A. Cronquist, K. Wymore, E. Booth, S. Lathrop, A. Palmer, and A. Bowen. 2014. Incidence of *Cronobacter* spp. Infections, United States, 2003–2009. *Emerg. Infect. Dis.* 20:9.
- Ray, P., A. Das, V. Gautam, N. Jain, A. Narang, and M. Sharma. 2007. *Enterobacter sakazakii* in infants: Novel phenomenon in India. *Indian J. Med. Microbiol.* 25:408.
- Reyad, S., O. Tareq, A. Wail, J. Ziad, and A. Mahmoud. 2007. Isolation of *Enterobacter sakazakii* and other *Enterobacter* sp. from food and food production environments. *Food Contr.* 18:1241–1245.
- Ribot, E. M., M. A. Fair, R. Gautam, D. N. Cameron, S. B. Hunter, B. Swaminathan, and T. J. Barrett. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathog. Dis.* 3:59–67.
- Sonbol, H., S. Joseph, C. M. McAuley, H. M. Craven, and S. J. Forsythe. 2013. Multilocus sequence typing of *Cronobacter* spp. from powdered infant formula and milk powder production factories. *Int. Dairy J.* 30:1–7.
- Stoop, B., A. Lehner, C. Iversen, S. Fanning, and R. Stephan. 2009. Development and evaluation of *rpoB* based PCR systems to differentiate the six proposed species within the genus *Cronobacter*. *Int. J. Food Microbiol.* 136:165–168.
- Thiruvankadan, A. K. 2012. Improvement in rural livelihood through dairy goat farming in India. Page 174 in First Asia Dairy Goat Conference, vol. 9. Universiti Putra Malaysia and the Food and Agricultural Organization of the United Nations, Rome, Italy.
- Townsend, S., E. Hurrell, and S. Forsythe. 2008. Virulence studies of *Enterobacter sakazakii* isolates associated with a neonatal intensive care unit outbreak. *BMC Microbiol.* 8:64.
- Xi, M., Y. Q. Feng, Q. Li, Q. N. Yang, B. G. Zhang, G. H. Li, C. Shi, and X. D. Xia. 2015. Prevalence, distribution, and diversity of *Escherichia coli* in plants manufacturing goat milk powder in Shaanxi, China. *J. Dairy Sci.* 98:2260–2267.
- Xu, X., Q. Wu, J. Zhang, Y. Ye, X. Yang, and X. Dong. 2014. Occurrence and characterization of *Cronobacter* spp. in powdered formula from Chinese retail markets. *Foodborne Pathog. Dis.* 11:307–312.
- Yan, Q. Q., O. Condell, K. Power, F. Butler, B. D. Tall, and S. Fanning. 2012. *Cronobacter* species (formerly known as *Enterobacter sakazakii*) in powdered infant formula: A review of our current understanding of the biology of this bacterium. *J. Appl. Microbiol.* 113:1–15.