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

Ruoyu Fang,*[1] Qianning Wang,†[1] Baowei Yang,* Jing Zhang,* Binyun Cao,‡ Wei Geng,§ Xianchao Feng,* Junjun Yang,* Jing Yang,# and Wupeng Ge*[2]
*College of Food Science and Engineering, Northwest A & F University, Yangling, Shaanxi 712100, China
†College of Bioengineering and Biotechnology, Tianshui Normal University, Tianshui, Gansu 741000, China
‡College of Animal Science Technology, A&F University, Yangling, Shaanxi 712100, China
§Xianyang Bureau of Quality and Technical Supervision, Xianyang 712000, China
#Institution of Inspection of Yangling Bureau of Quality and Supervision, Yangling 712100, China

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 ~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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[1]These two authors contributed equally to this work.
[2]Corresponding author: josephge@nwsuaf.edu.cn

(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 (Alférez et al., 2001; Haenlein, 2004; Mwenze and Kiplagat, 2011). China is the largest goat breeding and production country in the world (Luo, 2009; Thiruvenkadan, 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
Powder	Spray drying rooms	NT	1	1	2
	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
Swabs	Air filtration rooms	3	2	2	7
	Spray drying rooms	5	4	4	13
	Fluidized bed rooms	2	1	2	5
	Packaging rooms	6	6	7	19
	Total locations	25	25	25	75

[1]Not tested.