Short communication: Occurrence of *Arcobacter* species in industrial dairy plants

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ABSTRACT

The present study investigated the presence of *Arcobacter* spp. in industrial dairy plants. Between February and September 2013, pasteurized milk used for cheesemaking, processing and cleaning water, cheese, and environmental samples from different plant sites, including surfaces in contact or not in contact with food, were sampled. A total of 126 samples were analyzed by the cultural method and isolates were identified by multiplex PCR. *Arcobacter* spp. were isolated from 22 of 75 environmental samples (29.3%): of them, 22.7% were surfaces in contact with food and 38.7% surfaces not in contact with food. A total of 135 *Arcobacter* spp. isolates were obtained; of these, 129 and 6 were identified as *Arcobacter butzleri* and *Arcobacter cryaerophilus*, respectively. All food processing water and pasteurized milk samples were negative for *Arcobacter* species. We were not able to determine the primary source of contamination, but the isolation of both *A. butzleri* and *A. cryaerophilus* in surfaces in contact with food before and during manufacturing suggests that *Arcobacter* spp. are not or are only partially affected by routine sanitizing procedures in the industrial dairy plants studied. The efficacy of sanitizing procedures should be evaluated and further studies are needed to determine whether certain *Arcobacter* strains persist for long periods of time in industrial dairy plants and whether they can survive in different types of cheese in cases of postprocessing contamination.

Key words: *Arcobacter butzleri*, *Arcobacter cryaerophilus*, dairy plant, cheese

Short Communication

The *Arcobacter* genus has been linked to animal and human illness (Ho et al., 2006). In particular, the species *Arcobacter butzleri* and *Arcobacter cryaerophilus* have been associated with several cases of gastrointestinal disease, with persistent diarrhea as the main

symptom in humans (Collado and Figueras, 2011). A direct connection between consumption of *Arcobacter*-contaminated food and water and human illness has not yet been established, although it is likely that *arcobacters* are transmitted via these routes (González and Ferrús, 2011). *Arcobacter* spp. have been isolated from fecal samples of dairy animals (Piva et al., 2013; Shah et al., 2013) and found to contaminate different foods of animal origin, including milk (Ertas et al., 2010; Shah et al., 2012). Furthermore, 2 recent studies (Giacometti et al., 2013a,b) reported the first isolation of *A. butzleri* from environmental samples collected in an artisanal cheese factory, in a ready-to-eat cheese produced for retail, and in an industrial dairy plant. Given the scant data available to date, we investigated the occurrence of *Arcobacter* spp. in several industrial dairy plants.

The survey investigated the presence of *Arcobacter* spp. in 3 unrelated dairy plants (1 to 3) located in 3 different Italian regions. All dairy plants used only pasteurized cow milk for cheese production, and a high level of automation and good hygiene practices were in place. Dairy plants 1, 2, and 3 used a daily mean of 180,000, 445,000, and 120,000 kg of milk, respectively, per plant for cheesemaking.

Plant 1 had 2 completely separate production lines (A and B) producing, respectively, Mozzarella cheese and Stracchino cheese (a soft, creamy, unripened cheese that undergoes a short maturation period to obtain its typical texture). Plant 2 produced only Mozzarella cheese, whereas plant 3 produced Mozzarella cheese and Ricotta cheese (whey cheese). Plants 1 and 3 treated processing water by filtration and chlorination (1–1.5 ppm), whereas plant 2 treated the water by filtration, chlorination (1–1.5 ppm), and ozonization. All 3 plants performed a combination of cleaning-in-place and manual sanitation, using different sanitizing agents in different parts of the plants and production lines. Details of the sanitizing agents used are given in Table 1.

Sampling was done between February and September 2013. Plant 1 was sampled twice: in the first sampling, only the Mozzarella cheese line was considered, whereas the second sampling considered both lines (Mozzarella and Stracchino cheese lines); plants 2 and 3 were sampled once. A total of 126 samples were collected, placed

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Table 1. Sanitizing agents used in the 3 dairy plants by a clean-in-place (CIP) system or during manual sanitizing procedures, and their concentration of utilization as reported by the manufacturer

Plant	CIP	Manual sanitizing procedures
1	Hydrogen peroxide (0.1–0.5%)	Hydrogen peroxide, peracetic acid, and acetic acid (0.2–1%) Acetic acid, dodecylamine (2%)
2	Acetic acid, peracetic acid, hydrogen peroxide, and caprylic acid (1%)	Aminopropyl dodecylamine (1.5% for 20 min or 10% for 5 min)
3	Peracetic acid stabilized, hydrogen peroxide, and acetic acid (minimum 0.08%)	Hydrogen peroxide and citric acid (0.2–2%) Peracetic acid stabilized, hydrogen peroxide, and acetic acid (30 g/L)

in a Stomacher bag, and delivered to the laboratory in a portable refrigerator ($5 \pm 3^{\circ}\text{C}$). Samples included 12 samples of pasteurized milk used for cheesemaking, 12 processing water samples, 25 food samples, and 75 environmental samples; 2 additional samples were collected by swabbing toilet surfaces in plant 1. Environmental samples were obtained by sponging an area of approximately 250 cm^2 , representing the maximum area that could be sampled for all sampling sites. Sponging within the area consisted of 10 vertical passes (up and down was considered 1 pass) and 10 horizontal passes (side to side was considered 1 pass) for each large side of the sponge. Details of samples collected in each dairy plant are reported in Table 2.

Samples were processed within 2 to 24 h of collection and isolation was performed according to Houf et al. (2001): 25 mL of liquid samples or 25 g of solid samples and sponges were placed in a Stomacher bag and homogenized for 5 min in a stomacher in 225 mL of *Arcobacter* broth (Oxoid Ltd., Basingstoke, UK) supplemented with 5% laked horse blood (Oxoid Ltd.) and a mix of cefoperazone (16 mg/L), amphotericin B (10 mg/L), 5-fluorouracil (100 mg/L), novobiocin (32 mg/L), and trimethoprim (64 mg/L) as a selective supplement. All antimicrobial substances were obtained as laboratory standard powders from Sigma (St. Louis, MO). After 48 h of incubation at $28 \pm 1^{\circ}\text{C}$ under microaerobic conditions, a $10\text{-}\mu\text{L}$ aliquot of the enrichment broth was streaked onto selective agar plates prepared by suspending 24 g of *Arcobacter* broth (Oxoid Ltd.) and 12 g of Agar Technical No. 3 (Oxoid Ltd.) and supplemented with selective antibiotic mix supplement as described above. The plates were incubated microaerobically at $28 \pm 1^{\circ}\text{C}$ for 48 h. The microaerobic conditions were created by evacuating 80% of the normal atmosphere and introducing a gas mixture of 8% CO_2 , 8% H_2 , and 84% N_2 into the jar. Up to 10 colonies, if present, suspected to be *Arcobacter* spp. were picked from each plate, subcultured, and subjected to presumptive identification using tests that included Gram stain and growth under aerobic conditions and cellular morphology.

For identification of the presumptive *Arcobacter* colonies, the isolates were subjected to DNA extraction using the REDExtract-N-Amp Tissue PCR Kit (Sigma), and identified by the multiplex PCR described by Doudah et al. (2010); isolates identified as *A. butzleri* were confirmed by the multiplex PCR described by Houf et al. (2000). Two reference strains, *A. butzleri* DSM 8739^T and *A. cryaerophilus* DSM 7289^T (Leibniz Institute DSMZ, Braunschweig, Germany), were used as controls: they were grown separately on nutrient agar supplemented with 5% laked horse blood (Oxoid Ltd.) incubated microaerobically at 30°C for 48 h.

Table 2. Samples collected in the 3 dairy plants investigated and number and description of positive samples

Date	Plant	Type of sample (no.)	No. of <i>Arcobacter</i> - positive samples (%)	Description of (no.) positive samples and of <i>Arcobacter</i> species isolated		
				<i>Arcobacter</i> <i>butzleri</i>	<i>Arcobacter</i> <i>cryaerophilus</i>	<i>A. butzleri</i> and <i>A. cryaerophilus</i>
February 20, 2013	Plant 1, line A ¹	Surfaces in contact with food (9)	2 (22)	Curd conveyor (1); curd-cutting facilities (1)		
		Surfaces not in contact with food (5)	2 (40)	Floor drain (1); floor-cleaning facilities (1)		
April 10, 2013	Plant 1	Food (5)	0			
		Toilet swabs (2)	0			
		Processing water (3)	0			
		Pasteurized milk (3)	0			
		Surfaces in contact with food (4)	1 (25)			
	Plant 1, line A ¹	Surfaces not in contact with food (5)	0			
		Food (4)	0			
		Processing water (3)	0			
		Pasteurized milk (3)	0			
		Surfaces in contact with food (4)	1 (25)			
June 3, 2013	Plant 1, line B ²	Surfaces not in contact with food (6)	1 (17)			
		Food (1)	0			
		Processing water (3)	0			
		Pasteurized milk (3)	0			
		Surfaces in contact with food (4)	1 (25)	Molder (1)		
	Plant 2	Surfaces not in contact with food (6)	1 (17)	Plant access door (1)		
		Food (1)	0			
		Processing water (3)	0			
		Pasteurized milk (3)	0			
		Surfaces in contact with food (19)	4 (21)	Molder roller (1); packaging facilities (1)		
September 19, 2013	Plant 2	Surfaces not in contact with food (11)	5 (45)	Floor draining (4); sampler shoe covers (1)		
		Food (6)	0			
		Processing water (3)	0			
		Pasteurized milk (3)	0			
		Surfaces in contact with food (8)	2 (25)			
	Plant 3	Surfaces not in contact with food (4)	4 (100)			
		Food (9)	0			
		Surfaces in contact with food (8)	2 (25)	Curd-cutting facilities (1); curd conveyor (1)		
		Surfaces not in contact with food (4)	4 (100)	Floor draining (3); sampler shoe covers (1)		
		Food (9)	0			

¹Mozzarella cheese production line.

²Stracchino cheese production line.

Arcobacter spp. were isolated from 22 of 75 environmental samples (29.3%); namely, *Arcobacter* spp. were isolated from 12 of 31 surfaces not in contact with food (38.7%) and from 10 of 44 surfaces in contact with food (22.7%). All food (Mozzarella cheese, conditioning liquid, Stracchino cheese, and Ricotta cheese), processing water, and pasteurized milk samples were negative for *Arcobacter* spp. A total of 135 isolates were obtained; of these 129 and 6 were identified, respectively, as *A. butzleri* and *A. cryaerophilus*. The number and source of positive samples for each investigated dairy plant are detailed in Table 2.

Arcobacter spp. were isolated in all the cheese factories investigated and in all the samplings performed: *A. butzleri* was the most prevalent species isolated, namely 21 of 22 positive samples, whereas *A. cryaerophilus* was isolated only in 3 of the overall 22 positive samples, in agreement with previous studies performed in poultry and cheese-processing plants (Ferreira et al., 2013; Giacometti et al., 2013a) and also in milk (Scullion et al., 2006; Shah et al., 2012; Serraino et al., 2013). In addition, the fact that *A. butzleri* displayed a longer survival time and better growth ability than *A. cryaerophilus* in milk (Giacometti et al., 2014), was more resistant in several substrates (D'Sa and Harrison, 2005; Van Driessche and Houf, 2008), and that the enrichment step reduces the diversity of *Arcobacter* species by favoring the faster-growing species (Houf et al., 2002), could account for the more frequent isolation of *A. butzleri* than other species in both milk and in environmental samples.

Both *A. butzleri* and *A. cryaerophilus* were isolated from surfaces in contact with food before and during manufacturing in all the plants investigated and in all the samplings performed. This demonstrates that *Arcobacter* spp. are not or are only partially affected by routine sanitizing procedures in the industrial dairy plants studied, as previously demonstrated in poultry slaughterhouses (Houf et al., 2002, 2003; Ferreira et al., 2013), a spinach processing plant (Hausdorf et al., 2013), and an artisanal dairy plant (Giacometti et al., 2013a). A recent study reported that *A. butzleri* isolates were able to tolerate sodium hypochlorite concentrations close to working solutions used for sanitizing in food processing plants and, hence, may survive the disinfection process (Rasmussen et al., 2013). The recurrent finding of *Arcobacter* spp. on sanitized surfaces highlights a crucial issue for industrial plants and calls for further studies to investigate their ability to survive sanitizing procedures.

In this study, we were not able to determine the primary source of contamination because we excluded pasteurized milk and water contamination, different from a previous study (Giacometti et al., 2013a), which

identified raw milk used for cheesemaking as one of the prevalent sources of plant contamination.

To ensure safe food for consumers, food producers have a strong focus on hygiene during production. Cleaning and disinfection are the most common means of obtaining safe production environments and the presence of arcobacters, mainly *A. butzleri*, observed in the studied industrial dairy plants merits attention by food safety managers. On one hand, the observed *Arcobacter* spp. isolation from floors and floor drains, where they probably encounter favorable conditions for survival, may represent a source of spread through the plant by footwear and trolleys (as demonstrated by the isolation twice of *A. butzleri* from the shoe covers used by the sampler). On the other hand, the observed *Arcobacter* spp. isolation on food processing surfaces may represent a source of postprocessing food contamination.

In conclusion, the efficacy of sanitizing procedures against *Arcobacter* spp. should be evaluated and further studies are needed to establish whether certain *Arcobacter* strains persist for long periods of time in industrial dairy plants and whether they can survive in different types of cheese in cases of postprocessing contamination.

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