

Isolation of *Arcobacter butzleri* in environmental and food samples collected in industrial and artisanal dairy plants

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Abstract

This study investigated the presence of *Arcobacter* species in two cheese factories; a total of 22 environmental samples and 10 food samples were collected from an artisanal and an industrial cheese factory; *Arcobacter* species were isolated after enrichment, and isolates were identified at species level by multiplex-polymerase chain reaction (PCR) assay. In the artisanal cheese factory, *Arcobacter* spp. were isolated from several environmental samples, cow and water buffalo raw milk and ricotta cheese. In the industrial plant, *Arcobacter* spp. were isolated from surfaces not in contact with food and from a cleaned surface in contact with food; no *Arcobacter* spp. was isolated from food. All isolates were identified as *A. butzleri*. We report of the presence of *A. butzleri* in a ready-to-eat cheese produced for retail. In addition, the isolation of *A. butzleri* in food processing surfaces in the two cheese factories could be assessed as a source of potential contamination for cheeses.

Introduction

In recent years concern has grown over the genus *Arcobacter* because its members have been considered emergent enteropathogens and potential zoonotic agents (Collado and Figueras, 2011); interest in arcobacters in veterinary and human public health has increased from the first report of the isolation of arcobacters from food of animal origin. Since then, studies worldwide have reported the occurrence of arcobacters on food and in food production animals and have highlighted possible transmission, especially for *Arcobacter butzleri*, to the human population

(Doudiah *et al.*, 2012).

Potential routes of *Arcobacter* spp. transmission to humans are the consumption of contaminated foods of animal origin and water (Shah *et al.*, 2011). The initial source seems to be fecal contamination during the various stages of production processes (Van Driessche and Houf, 2008).

Arcobacter butzleri is the most important and prevalent species of the genus: it has been classified as a serious hazard to human health by the International Commission on Microbiological Specifications for Foods (ICMFS, 2002) and as a significant zoonotic pathogen (Cardoen *et al.*, 2009). Few surveys have investigated the presence of *Arcobacter* spp. in bulk tank cow raw milk and the reported prevalence rates were 46% in Northern Ireland (Scullion *et al.*, 2006), 5.8% in Malaysia (Shah *et al.*, 2012) and 48% in Italy (Milesi, 2010); in Italy, *A. butzleri* was isolated in fecal samples and in line milk filters in a water buffalo dairy farm (Piva *et al.*, 2013; Serraino *et al.*, 2013). Hitherto no data were reported on isolation of *A. butzleri* in dairy products. Considering the *A. butzleri* ability to form biofilm and to survive in food processing plants (Ferreira *et al.*, 2013), no available data are in literature on its distribution in the food plants environment with the exception of poultry slaughterhouses. For these reasons, in this study, we have investigated the presence and distribution of *Arcobacter* spp. from food and environment of two dairy plants, respectively in one artisanal and in one industrial.

Materials and Methods

The study was carried out on two dairy plants: an artisanal one which produces raw milk water buffalo (WB) *mozzarella* cheese and cheeses made from pasteurised cow milk and an industrial dairy plant which produces *mozzarella* made from pasteurised cow milk; both dairies produces also *ricotta* cheese. Food and environmental samples were collected in a single day in each of the two dairies. Environmental samples were collected from surfaces in contact with food during operation, surfaces not in contact with food (for example the floor) and surfaces in contact with food before use (cleaned surfaces) by swabbing at least 250 cm² when possible; the following food samples were collected in the two dairies, respectively raw WB milk, raw cow milk, WB *mozzarella* cheese, WB *ricotta* cheese and the conditioning liquid in the artisanal dairy plant, and *mozzarella* cheese and conditioning liquid in the industrial dairy plant; in both dairies, a total of two samples of tap water used during

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food processing were collected. A total of 22 swabs, 10 food samples and 4 water samples were collected (more details are reported in Table 1). All the samples were placed in a sterile bag, transported to the laboratory in refrigerated coolers at 5±3°C, and processed within 4 h of sampling. Isolation was performed using the enrichment procedure described by Houf *et al.* (2001); briefly, each sample, respectively swab or 25 mL of liquid sample or 25 g of solid sample, was put into *Arcobacter* broth (Oxoid Ltd., Basingstoke, UK) supplemented with 5% lysed horse blood (Oxoid Ltd.) and a mix of cefoperazone (16 mg/L), amphotericin B (10 mg/L), 5-fluorulacil (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, USA). After 48 h of incubation, an aliquot of 10 µL 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 supplement as described above. The plates were incubated at 28±1°C under microaerobic conditions and after 48 h of incubation were checked daily up to 5 d. Colonies of Gram negative spiral bacteria were subcultured and subjected to presumptive identification using tests that included growth under aerobic conditions, cellular morphology. Colonies presumptive for *Arcobacter* spp. were subjected to DNA extraction using REExtract-N-Amp tissue PCR Kit (Sigma), and identified by the multiplex-polymerase chain reaction (PCR) described by Doudiah *et al.* (2010).

Table 1. Number of samples performed and number of positive samples for *A. butzleri* in an artisanal and industrial cheese factory.

Type of sample	Artisanal cheese factory		Industrial cheese factory			
	Description	N	P	Description	N	P
Swab on cleaned food contact surfaces	Draining table, <i>mozzarella</i> cheese molding roller,* curd cutter	3	1	Curd cutting facilities,* <i>mozzarella</i> cheese molding roller	2	1
Swab on food contact surfaces during operation	Bulk tank valve,* cheese vat,* milk pump	3	2	<i>Mozzarella</i> cheese molding roller (2), internal surface of cooling vat (2), <i>mozzarella</i> cheese packing machine, cheese vat, curd conveyor*	7	1
Swab on non-food contact surfaces	Cooler room floor* floor draining*	2	2	Floor draining (2),* shovel, external surface of pipes, external surface of <i>mozzarella</i> cheese cooling vat	5	1
Food	Raw cow milk,* raw WB milk,* <i>ricotta</i> cheese,* <i>mozzarella</i> cheese, <i>mozzarella</i>	5	3	Pasteurised milk, <i>mozzarella</i> cheese, <i>mozzarella</i> cheese conditioning liquid (2), <i>ricotta</i> cheese	5	0

N, number of samples performed; P, number of positive samples. *Positive sample for *A. butzleri*; numbers in brackets indicate number of samples analysed.

Results

Arcobacter spp. were isolated respectively from several environmental, cow and WB raw milk and *ricotta* cheese samples in the artisanal cheese factory and, in the industrial plant, from surfaces not in contact with food, in contact with food during operation and from a cleaned surface in contact with food. No *Arcobacter* spp. was isolated from food in the industrial plant. Details on the number of positive samples are reported in Table 1. All the isolates have been identified as *A. butzleri*.

Discussion

We report the isolation of *Arcobacter* spp. from environmental samples collected in cheese factories and in ready-to-eat cheese produced for retail. The results of this study should be interpreted carefully as they were obtained from a single investigation for each dairy plant; nevertheless some considerations are required.

Firstly, *A. butzleri* was recovered most frequently from the environmental samples collected in the artisanal cheese factory than in the industrial cheese factory; to our knowledge this difference could be related to the fact that: i) in the artisanal cheese factory both pasteurised and raw milk are used for cheese manufacturing whereas in the industrial cheese factory only pasteurised milk is used and therefore the possibility that *A. butzleri* is imported into the dairy environment by contaminated milk is negligible (Scullion *et al.*, 2006; Milesi, 2010; Shah *et al.*, 2012); ii) the high level of automation and the layout or hygienic design in the industrial cheese facto-

ry processing reduces the probability of cross contaminations due to worker activity; iii) the improved sanitizing procedures, that also included a cleaning-in-place system in the industrial cheese factory whereas are performed by the workers in the artisanal cheese factory. In the artisanal plants these aspect enhance the probability of misapplication of the good sanitation practices. Given the demonstrated ability of *A. butzleri* to form biofilm (Kjeldgaard *et al.*, 2009; Ferreira *et al.*, 2013), the presence of *A. butzleri* in cleaned processing surfaces must be taken into account as source of post processing contamination. With the results of this study we could not assess if the observed environmental contamination in the two cheese factories is due to biofilm formation, but the contemporary presence of *A. butzleri* in different areas and in different type of surfaces (*i.e.* in contact or not with food and samples collected from cleaned food processing surfaces or during operation) suggests that milk is not the only source of cheese contamination.

Conclusions

The isolation of *A. butzleri* in the *ricotta* cheese produced in the artisanal cheese factory confirms the food processing surfaces as source of contamination of *A. butzleri* for dairy products; in fact, in the investigated dairy plant the *ricotta* cheese is produced by direct steaming of whey up to 90°C and it is unlikely that *A. butzleri* will survive the thermal treatment (D'Sa and Harrison, 2005; Hilton *et al.*, 2001). Furthermore, after surfacing step, *ricotta* cheese is put by a ladle in moulds on a steel draining table and the contact with these surfaces, given the demonstrated presence of *A.*

butzleri, could be assumed as the most probable source of *ricotta* cheese contamination. Consequently, the isolation of *A. butzleri* in food processing surfaces in the two cheese factories represents a source of potential contamination for cheeses.

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