*Arcobacter butzleri* in dairy plants

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

**Isolamento di *Arcobacter* *butzleri* in campioni ambientali ed alimentari in un caseificio industriale ed artigianale**

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**Riassunto**

Il presente studio ha valutato la presenza di specie di *Arcobacter* in due caseifici; ventidue campioni ambientali e dieci campioni alimentari sono stati raccolti presso un caseificio artigianale ed uno industriale; la ricerca di *Arcobacter* spp. è stata effettuata tramite arricchimento e gli isolati sono stati identificati mediante multiplex-PCR. Ceppi di *Arcobacter* spp. sono stati isolati, nel caseificio artigianale, in numerosi campioni ambientali, di latte crudo vaccino e bufalino e ricotta mentre, nello stabilimento industriale, in alcuni campioni di superfici a contatto e non a contatto con alimenti; nessun *Arcobacter* spp. è stato rilevato in campioni alimentari. Tutti gli isolati sono stati identificati come *A. butzleri*. Il presente studio è il primo report della presenza di *A. butzleri* in un formaggio pronto per la vendita al dettaglio; l'isolamento di *A. butzleri* in superfici di lavorazione di entrambi i caseifici potrebbe essere una potenziale fonte di contaminazione per la produzione di formaggi.

**Abstract**

This study investigated the presence of *Arcobacter* species in two cheese factories; a total of twentytwo environmental samples and ten 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 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*. To our knowledge this is a first report of the presence of *A. butzleri* in a ready to eat cheese produced for retail and, 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.

**Keywords**: *Arcobacter butzleri*, Dairy plant, Environment, Cheese.

**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 (Douidah 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 pasteurized cow milk and an industrial dairy plant which produces mozzarella made from pasteurized 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 food processing were collected. A total of 22 swabs, 10 food samples and 4 water samples were collected and 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 four hours 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.) 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). 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 REDExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, USA),and identified by the multiplex PCR described by Douidah et al. (2010).

**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 and conclusions**

This is the first report of 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 pasteurized and raw milk are used for cheese manufacturing whereas in the industrial cheese factory only pasteurized milk is used and therefore the possibility that *A. butzleri* is imported into the dairy environment by contaminated raw milk is negligible (Scullion et al., 2006; Milesi, 2010; Shah et al., 2012); ii) the high level of automation in the industrial cheese factory processing reduces the probability of cross contaminations due to worker activity; iii) the sanitizing procedures are performed by a Cleaning In Place system in the industrial cheese factory whereas are performed by the workers in the artisanal cheese factory: this aspect enhances the probability of misapplication of the good sanitation practices and, 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.

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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|  | **Artisanal cheese factory** | | | **Industrial cheese factory** | | |
| **Type of sample** | **Description** | **N** | **P** | **Description** | **N** | **P** |
| **Swab on cleaned food contact surfaces** | draining table, mozzarella cheese molding roller\*, curd cutter | 3 | 1 | curd cutting facilities\*, mozzarella cheese molding roller | 2 | 1 |
| **Swab on food contact surfaces during operation** | bulk tank valve\*, cheese vat\*, milk pump | 3 | 2 | mozzarella cheese molding roller (2), internal surface of cooling vat (2), mozzarella 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 mozzarella cheese cooling vat | 5 | 1 |
| **Food** | raw cow milk\*, raw WB milk\*, ricotta cheese\*, mozzarella cheese, mozzarella cheese conditioning liquid | 5 | 3 | pasteurized milk, mozzarella cheese, mozzarella cheese conditioning liquid (2), ricotta cheese | 5 | 0 |

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

\*positive sample for *A. butzleri*

numbers in brackets indicate number of samples analyzed