# Survival of selected bacteria from the genus *Arcobacter* on various metallic surfaces

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**Summary**

The genus *Arcobacter* is closely related to the well-known human pathogen *Campylobacter jejuni*. These bacteria are today dreadful pathogens and constitute a considerable threat, particularly in the form of alimentary infection. Many materials and surfaces, in particular those used in food industry, have been found to influence survival of bacteria, and can even lead to elimination of bacteria. The survival of selected arcobacters was tested on various metallic coupons at various temperatures. Arcobacters survived for the longest on stainless steel and on aluminium, but depending on the species of bacteria and on temperature (at 5 °C, for 8 h or 24 h, respectively; at 25 °C, for 120 min or 180 min, respectively). The substantially fastest inactivation was recorded in the case of materials containing copper or zinc. Inactivation occurred on the copper coupon already 3 min after exposure. On material coated with zinc, bacteria survived for 5 min or 10 min. This study is the first dedicated to such extent to the survival of arcobacters on these materials under various temperatures. Results also demonstrate a noteworthy sensitivity of arcobacters to this effect in comparison with several previously published results for other pathogenic bacteria.

**Keywords:**

*Arcobacter*; metallic surfaces; survival; alloy; cross-contamination

Bacterial infection from food continues to play a role in the high occurrence of various diseases. Bacteria of the genus *Campylobacter* are currently regarded to be among the main causes of alimentary diseases [1, 2]. The closely related bacterial genus *Arcobacter* was first described in 1991 [3, 4]. These bacteria are Gram-negative, slender, spiral-shaped rods and, along with the genus *Campylobacter*, belong to the family *Campylobacteraceae* [5]. A number of new species have in recent years been classified into this genus, which currently includes 17 species. Many of these species have been isolated from poultry [6], meat, feces of humans and animals suffering from gastrointestinal tract disease, and from aborted cattle fetuses (*A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A. cibarius*, *A. thereius*, and *A. trophiarum*). Recently, however, they are often found also in samples from marine environments (*A. marinus*, *A. mollusorum*, *A. mytili*, *A. ellisii*, *A. bivalviorum*, and *A. venerupis*) [7–9]. Other representatives include *A. defluvii* from sewage water, *A. nitrofigilis* and *A. halophilus* from salt marshes [8], and the newly classified representatives *A. cloacae* and *A. suis* [9].

Arcobacters are aerotolerant and are able to grow at lower cultivation temperatures than are campylobacters [6]. The optimal conditions for arcobacters are the presence of oxygen in microaerobic conditions and temperature of 15–30 °C [10]. Growth has been observed also at lower temperatures and even at 5 °C, which is the temperature of, for example, underground water [11]. Survival at refrigerator temperatures is often the cause for contamination of food [12]. Studies have documented the frequent presence of *Arcobacter* spp. in the poultry slaughterhouse environment, on the along equipment of the processing line, including the processing water [13, 14], on

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chicken carcasses at different processing stages of slaughter [15], and at on the retail level [6]. The prevalence of *Arcobacter* spp. on poultry products is the highest and can range between 24% and 96% [16].

The effect of desiccation on various materials is today one of the extensively studied influences in the struggle against undesirable microflora [17–22]. As a result of desiccation, cell vitality is temporarily influenced and cells transit into viable but non-culturable form. This is followed by full cessation of metabolic processes and then cell death [23]. Sensitivity to desiccation varies in bacteria and also varies depending on many additional environmental parameters. It is generally accepted that bacteria are able to survive on various materials depending on their tolerance to desiccation and also depending on the type and composition of the material [21, 23].

For example, cupriferous materials are known for their inhibitory effect on a range of microorganisms in comparison with plastic, stainless steel, and other types of surfaces [21]. However, poor resistance and softness of cupriferous materials represent disadvantages for their routine use in the food processing industry [22].

Stainless steel is a commonly used material for work areas in the food processing industry. This material is relatively inert to microorganisms and, in case of their possible inhibition, likely contributes to the desiccation effect. Nevertheless, microflora can survive for a relatively long time on this surface and contaminated surfaces can thus be potentially unsafe during the production and processing of food [21].

Several studies have investigated the potential risk of surface contamination in food processing environments. Contamination often occurs between raw and cooked foodstuffs and by the contact of cooked produce with contaminated surfaces [17, 22, 24]. In recent years, many antimicrobial agents and surfaces, such as metal oxides and various metallic surfaces, have received increasing attention in food applications. They are stable under high temperatures and pressures that may occur in harsh food-processing conditions, but they are also generally regarded as safe for humans and animals [25, 26].

This study focuses on the inhibitory effect of various metallic materials in relation to bacteria of the genus *Arcobacter*, which can constitute a significant risk for food contamination. The study describes, for the first time, high sensitivity of arcobacters to several metallic materials at various temperatures.

## MATERIALS AND METHODS

### Bacterial cultures and cultivation

The following cultures were used: *Arcobacter butzleri* CCUG 30484 from the Culture Collection (University of Göteborg, Göteborg, Sweden); *Arcobacter cryaerophilus* CCM 7050 from the Czech Collection of Microorganisms (Masaryk University, Brno, Czech Republic) and *Arcobacter defluvii* LMG 25694 from the Belgian Co-ordinated Collection of Microorganisms (University of Ghent, Ghent, Belgium).

All cultures were cultured on tryptone soya agar (TSA; Himedia, Mumbai, India) under aerobic conditions for 48 h at 30 °C and then stored at 4 °C. Bacterial suspensions were prepared at a density of ~$10^8$ CFU·ml$^{-1}$ (McFarland Turbidity Standard No. 0.5; Thermo Fisher Scientific, Brno, Czech Republic) in physiological saline solution (0.85% of NaCl in distilled water) and subsequently, serial decimal dilutions were prepared in physiological solution (final density, $10^6$ CFU·ml$^{-1}$).

### Optimization of experimental methods

Three methods were tested for transferring cells from a stainless steel coupon into physiological solution and their quantitation after cultivation on agar medium. The experiment was conducted first with *A. butzleri* CCUG 30484, after which verification was conducted with *E. coli* bacteria (data not shown).

A total of 20 μl of cell suspension with a density of $10^6$ CFU·ml$^{-1}$ were pipetted onto a 1 cm × 4 cm sterile coupon. The cells were then transferred from the surface of the coupon into 10 ml of physiological solution, with the help of ultrasound (coupon transferred into physiological solution and left for 1 min in a 38 kHz ultrasonic bath), being wiped with a sterile swab (coupon swabbed 10 times and the swab shaken in a vortex for 1 min), and with the help of a vortex (coupon transferred into physiological solution and immediately vortexed for 1 min). A volume of 100 μl of the suspension thus obtained was inoculated onto the surface of TSA agar and cultured for 48 h at 30 °C. The actual density of the initial cell suspension was verified by cultivation method on TSA agar after appropriate dilution (final density $10^2$ CFU·ml$^{-1}$).

### Determination of the survival of arcobacters on metallic surfaces

The survival of *A. butzleri* CCUG 30484, *A. cryaerophilus* CCM 7050 and *A. defluvii* LMG 25694 was tested on coupons consisting of

selected metallic materials (stainless steel, aluminium, copper, brass, and galvanized material – zinc). All coupons were first cleaned with a detergent, washed with distilled water, wiped with ethanol, then sterilized in a dry heat sterilizer Sterimat (BMT, Brno, Czech Republic). During the experiment, coupons were placed in sterile plastic Petri dishes to eliminate contamination from the environment.

A volume of 20 $\mu$l of the cell suspension was pipetted onto each tested coupon and the coupons were transferred into test tubes with 10 ml of physiological solution after a monitored period of exposure (0–180 min; 0–24 h). The test tubes were then homogenized for 1 min using a vortex.

A 100 $\mu$l aliquot of each sample was transferred onto the surface of TSA agar and incubated for 48 h at 30 °C. The dependence of the number of cultivated cells upon time elapsed was observed (at 5 °C and 25 °C).

As a control for the density of cells actually introduced onto the material, processing was always conducted at time $t_0$.

Immediately after applying the suspension, the coupon was transferred into a test tube with physiological solution and then processed as described above.

After each exposure time, it was determined whether the cells were actually dead or in viable-but-nonculturable (VBNC) form. This was done by cultivation of the coupon in Brain-heart infusion (BHI) broth (Himedia) under aerobic conditions for 48 h at 30 °C. Simultaneously, a control of actual cell density was always carried out by inoculating 100 $\mu$l of the cell suspension appropriately diluted in physiological solution. Control curves of the survival without the effect of materials were determined.

All experiments were repeated in at least triplicate, and the means and standard deviations were calculated for the results. The actual density of the initial cell suspension was verified by culture on TSA after appropriate dilution (final density $10^2$ CFU·ml$^{-1}$).

## RESULTS AND DISCUSSION

### Optimization of the method for transferring cells from the coupon into liquid media

Various techniques for quantification of surviving as well as cultivated cells were compared in order to determine experimentally the survival of arcobacters on metallic surfaces.

The most suitable method was determined to be the approach involving inoculation of the cell suspension onto a coupon that, after a monitored period of exposure, was transferred aseptically into a test tube with 10 ml of physiological solution and the contents of which were vortexed for 1 min in order to release the cells from the coupon. The highest numbers of cells were recorded using this method. The differences between the individual methods were observed from the beginning of the experiment. The average numbers of cells at, for example, time $t_0$ were as follows: 275 CFU (method using the vortex), 149 CFU (method using ultrasound), and 65 CFU (swab method), from the identical original density of $10^6$ CFU·ml$^{-1}$ inoculated onto the coupon. In all cases, *A. butzleri* CCUG 30484 cells were inactivated after 120 min of exposure. The results of these experiments are presented in Fig. 1.

In a similar study, CERVENKA et al. [27] applied a method with ultrasound. While we determined, through comparison of individual methods, that this method may also be used in this case, more accurate results were in fact achieved through the method using a vortex.

The swab method, meanwhile, was determined to be the least accurate. In the latter case, the cells were not sufficiently removed from the swab and, therefore, the numbers of cells were underestimated. This was verified by inoculating the cultivation medium with a swab after it had been shaken. The swab method is nevertheless widely used, for reasons of practical feasibility [6].

According to our results, the most suitable method was determined to be the one using a vortex. This method was previously used in several similar studies [19, 21, 22].

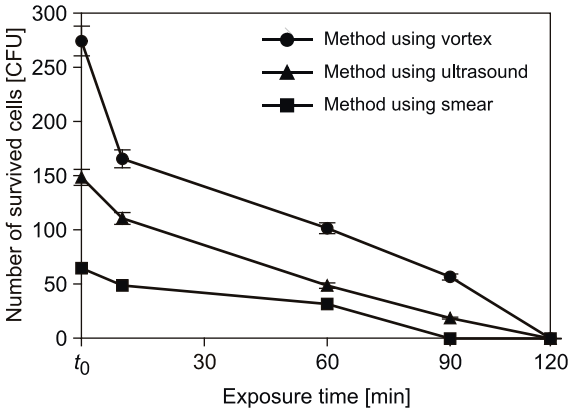



**Fig. 1.** Transfer of bacterial cells from the coupon into the solution by different methods.

Survival of *A. butzleri* CCUG 30484 on stainless steel at 25 °C was monitored. Results are means ($n$ = 3), bars represent standard deviations.

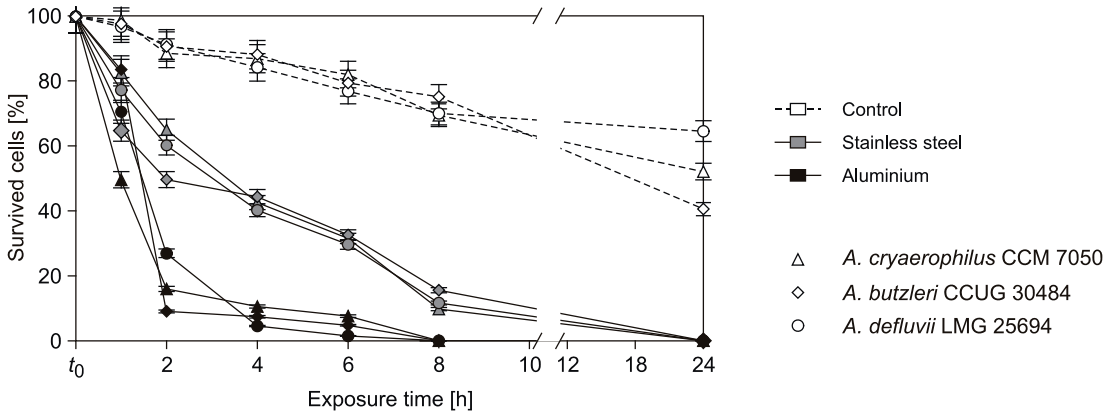



**Fig. 2.** Inactivation of arcobacters over time at 5 °C on various materials.

Results are means ($n$ = 3), bars represent standard deviations.

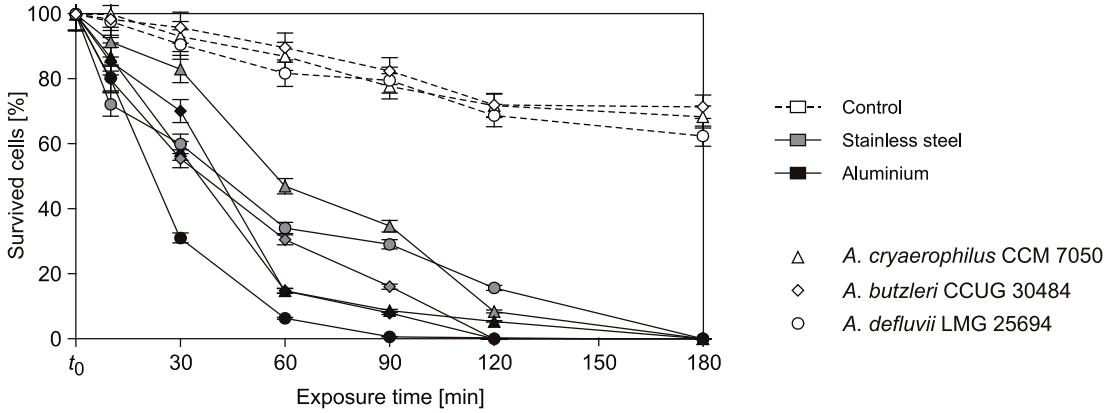



**Fig. 3.** Inactivation of arcobacters over time at 25 °C on various materials.

Results are means ($n$ = 3), bars represent standard deviations.

**Survival of arcobacters on metallic surfaces**

This study shows the antimicrobial effect of materials against bacteria of the genus *Arcobacter*. The experiments were conducted at room temperature (25 °C) and some also at a refrigerator temperature (5 °C). The experimental results obtained clearly indicated that inhibition of arcobacters was different on various surface materials and at different temperatures.

At 5 °C, complete inactivation of the tested species of the genus *Arcobacter* was observed after 24 h on stainless steel and after 8 h on aluminium (Fig. 2). Inactivation of all tested arcobacters had essentially the same trend for a given material. In the case of inert stainless steel, survival after 2 h was 65.1% ± 1.4% (*A. cryaerophilus*), 60.3% ± 2.0% (*A. defluvii*), and 49.7% ± 4.6% (*A. butzleri*) of the original cells. A more significant decrease in surviving cells was recorded in the case of aluminium. In this case, after the same period, 16.0% ± 3.6%, 27.0% ± 3.5%, and 9.0% ± 1.5% of the originally inoculated cells survived.

Inactivation of arcobacters was significantly faster at 25 °C (room temperature) and became complete after 120 min or 180 min of exposure (Fig. 3). Differences could be noticed among individual species. *A. butzleri* CCUG 30484, which was already inactivated after 120 min, seemed more sensitive to desiccation. By contrast, *A. cryaerophilus* survived longer on aluminium and was fully inactivated only as late as after 180 min.

Additional experiments monitored inhibition of arcobacters on copper, brass and a galvanized material (zinc). The results showed a strong inhibitory effect of these materials (Fig. 4). According to our results, all cells were dead after a final exposure time of our experiments (no growth in BHI broth).

Stainless steel is a material widely used in environments with a risk of bacterial contamination. This material is often preferred due to its durability, ease of cleaning and resistance to chemicals

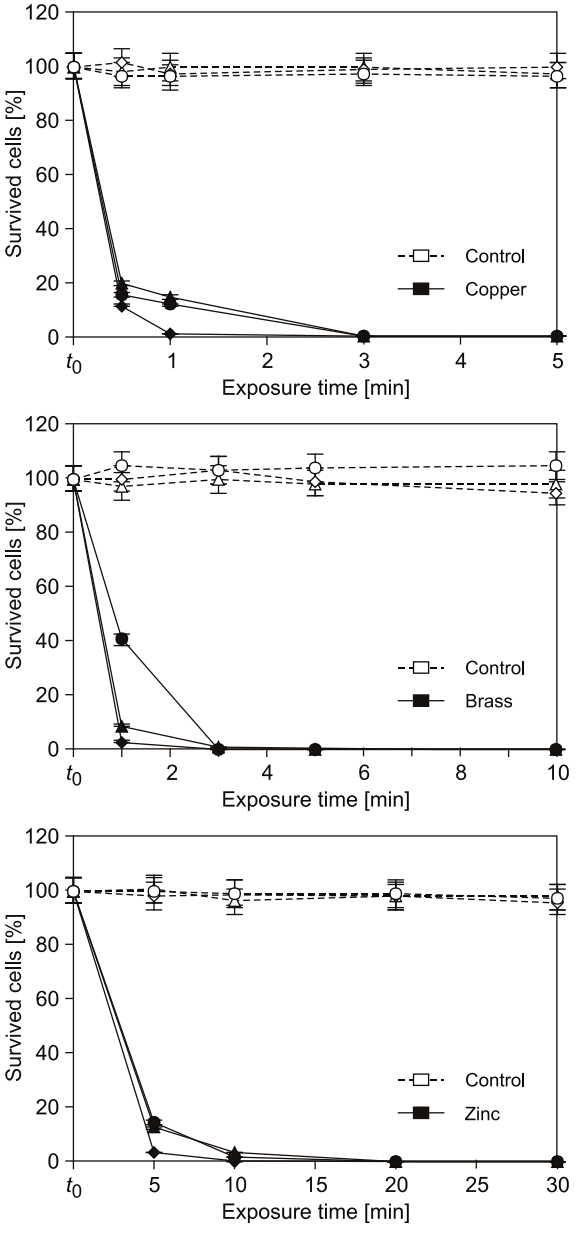
and corrosion. Nevertheless, previous studies indicated that it is not advantageous material in terms of its absence of antimicrobial properties [21, 28]. WILKS et al. [21] established that *E. coli* O157 was able to survive the desiccation process for more than 28 days on this surface at both room and refrigerator temperatures. The authors described a 5-log reduction in live cells occurring during the first 2 days, but then the density remained at a constant level of $10^4$ CFU·ml$^{-1}$. In our study with bacteria of the genus *Arcobacter*, significantly lower survival periods were established. Complete inhibition occurred on stainless steel after 120 min or 180 min at room temperature (according to species) and after 24 h at 5 °C. Our results constitute a significant difference in comparison with *E. coli*, and demonstrate high sensitivity of arcobacters to desiccation. Similar short periods of survival of arcobacters were also observed in a previous study by a different method [27].

Inhibition of arcobacters on aluminium at 5 °C was faster in comparison with stainless steel, with complete inactivation occurring after 8 h and with a more rapid decline in numbers of surviving cells during the observation period. At room temperature, cell inactivation occurred after 120 min, or after 180 min in the case of a more resistant strain *A. cryaerophilus* CCM 7050. More significant differences with stainless steel were not recorded at this temperature. A longer survival period at lower temperatures could be expected based on the knowledge of bacterial physiology under environmental conditions [29].

Materials containing copper are known to reduce the numbers of bacteria, as copper has a strong antimicrobial effect against a range of bacteria [21, 22]. Nevertheless, copper alone cannot be a completely suitable alternative to other materials, as it is an unstable material. There are, however, alloys containing copper that have significantly better properties for use in certain environments than copper alone [21].

In our study, arcobacters were inactivated very quickly both on copper alone and on brass alloy. Essentially, it may be stated that no live cells were found on these surfaces containing copper after 3 min of exposure at temperatures of both 5 °C and 25 °C, not even after subsequent resuscitation in a culture medium. This once again demonstrated the substantial sensitivity of arcobacters to environmental influences. According to the results of WILKS et al. [21], *E. coli* O157 did not survive for 90 min on an alloy containing copper. Similar conclusions emerged from other studies on the survival of methicillin-resistant strains of *Staphylococcus aureus* on cupriferous surfaces [19]. Arcobacters are highly sensitive in comparison with those bacteria.

Materials containing zinc are also known to have antimicrobial activity against Gram-positive and Gram-negative microflora, including *E. coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, *Staph. aureus* and *C. jejuni* [30–32]. To date, however, there has not been sufficient data that




△ *A. cryaerophilus* CCM 7050 ◇ *A. butzleri* CCUG 30484
○ *A. defluvii* LMG 25694

**Fig. 4.** Inactivation of arcobacters on copper, brass and galvanized (zinc) materials.

Results are means ($n$ = 3), bars represent standard deviations.

would demonstrate the influence of zinc on other important food pathogens from the genus *Arcobacter*.

Our study shows that *A. cryaerophilus* and *A. defluvii* became completely inhibited after 20 min of exposure on galvanized (zinc) material. *A. butzleri* was once again the most sensitive, and cells were devitalized after 10 min on this surface.

## CONCLUSIONS

This study demonstrates the sensitivity of arcobacters to various metallic surfaces made from materials applied in food industry. Bacteria of the genus *Arcobacter* could survive for a relatively long time on stainless steel under certain conditions. However, the survival of these bacteria was much shorter on cupriferous materials. Certain reduction in the survival time was observed also on materials containing zinc.

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