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SURVIVAL CAPACITY OF *Arcobacter butzleri* AT DIFFERENT INCUBATION TEMPERATURES IN DRINKING WATER

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Abstract

Arcobacter represents a zoonotic emerging pathogen with increasing importance for public health and drinking water has been cited as a major risk factor for its dissemination. The aim of this work was to evaluate the survival capacity of *Arcobacter* in different water matrixes stored at different temperatures. Three different water matrixes were used, including potable water with a chlorine concentration of 0,5 mg/mL, non-chlorinated water and non-chlorinated water added with an 11% of organic matter. Each matrix was inoculated in a 1/10 proportion with 103 and 105 *Arcobacter* pools, divided into 4 different subsamples, in order to be incubated at 0°C, 5°C, 12 °C and 25°C by up to 15 days. The presence of *Arcobacter* in each matrix was determined on days 1, 3, 5, 7, 9, 11, 13 and 15. Results obtained show that this bacterium can survive in all the water matrixes evaluated, regardless of the presence or not of residual disinfecting agent. Also, the amount of CFU/mL inoculated in water correlates with the number of bacteria that can survive on it, and that incubation temperature has a significant effect over the bacterial survival.

Keywords: Arcobacter butzleri. Drinking water. Survival.

1. Introduction

Arcobacter is a zoonotic emerging pathogen with increasing importance due to the risk it represents for human and animal health and the economic losses it can cause. Arcobacter can be the cause of gastroenteritis in human beings, and some species, especially Arcobacter butzleri, have been associated even with bacteremia, the reason why it has been included in the list of dangerous microorganism for human health by the International Commission of Microbiological Specifications for Food (ICMSF) (Banting and Figueras 2017).

This bacterium has also been associated with mastitis in cattle, gastric ulcers in pork, reproductive problems in production animals and its prevalence in animal derivates has been described as high (Collado and Figueras 2011).

Arcobacter butzleri is also a widely distributed microorganisms, and water is a likely key component of its transmission. It has been isolated from different environments including lakes, seawater, subterraneous water and even muds and sludge (Moreno et al. 2004). It has also been recovered from

wastewater, superficial water bodies and even potable water, indicating that this is an important transmison source (Lee et al. 2012).

Drinking water has been cited as a major risk factor for the dissemination of this bacterium (Moreno et al. 2004). Arcobacter has been associated with at least four different waterborne outbreaks. Two were linked to contaminated well water, one to a drinking water network connected to a new building, and the last one due to a distribution pipe breakage (Rice et al. 1999; Jalava et al. 2014; Laishram et al. 2016).

This bacterium has been shown to possess the ability to form biofilms, which greatly impacts its survival capacities on abiotic surfaces (Giacometti et al. 2015). This characteristic, together with its wide distribution, resistance to disinfecting agents and survival as endocytobiont inside free-living amoebas are some of the reasons why water is considered as one of their main transmission routes (Villanueva et al. 2016; Girbau et al. 2017).

Several studies have demonstrated a direct transmission of Arcobacter through water consumption both for human beings and animals and especially in developing countries, where some deficiencies in potable water treatment might occur (Assanta et al. 2002). Nevertheless, a growing controversy has been developed, since it has also been isolated from completely potable water in developed countries (Girbau et al. 2017). Because of this, it is important to evaluate the survival capacity of Arcobacter in different water matrixes stored at different temperatures, in order to determine the real risk this bacterium may represent for public health. The hypothesis for this research is that Arcobacter butzleri survival will be greater in non-chlorinated water matrix than in chlorinated matrix, being the aim of this study the evaluation of the survival capacity of Arcobacter butzleri in different water matrixes stored at different temperatures, in order to determine the real risk this bacterium may represent for public health.

2. Material and Methods

Inoculum preparation

Five different strains of Arcobacter butzleri, previously isolated from poultry fecal samples and identified through the Douidah's multiplex-PCR assay (Valverde-Bogantes et al. 2015) were used as inoculum source. These strains were grown on blood agar. A pool with two different concentrations, 103 and 105 CFU/mL was prepared in order to inoculate the water matrixes to be tested.

Inoculation and incubation

Three different water matrixes were used, including potable water with a chlorine concentration of 0.5 mg/mL, obtained from tap water at the Water Microbiology Lab, University of Costa Rica and supplied by the Instituto Costarricense de Acueductos y Alcantarillados (AyA). The second matrix was non chlorinated water obtained from a water well and the third matrix was non chlorinated water added with 11% of organic matter. For chlorinated water, free chlorine concentration was determined using the free chlorine colorimeter Cheker Hanna HI701.

Before conducting survival tests, each water matrix was analyzed in order to discard the presence of Arcobacter, using the broth enrichment culture method, followed by the passive membrane filtration technique on blood agar (Valverde-Bogantes et al. 2015), all being Arcobacter negative.

Each matrix was inoculated in a 1/10 proportion with the bacteria pools described above. Afterwards, each water matrix was divided into 4 different subsamples, in order to be incubated at 0°C, 5°C, 12 °C and 25°C for up to 15 days. The presence of Arcobacter in each matrix was determined on days 1, 3, 5, 7, 9, 11, 13 and 15. Briefly, after each incubation period, 100 ul of each matrix were streaked over blood agar plates. These were incubated aerobically, at 26°C for 72 h. Survival was confirmed by typical colony morphology and Gram staining. Assays were performed in duplicate.

Statistical analysis

Results obtained were analyzed using the Minitab 18 statistical software. A statistical comparison was carried out to determine whether there was statistical difference between the different water matrixes tested. The chi-square test was applied with a 95% confidence level.

3. Results

Tables 1-4 describe the survival capacity of Arcobacter butzleri through time and incubated at different temperatures. At 0°C, there was no survival of either inoculum tested in chlorinated water matrix.

For non-chlorinated water matrix and non-chlorinated water + organic material, survival was slight, and it did not last more than 3 days as shown on Table 1. The results obtained at 5°C were similar to that obtained at 0°C (Table 2). Results obtained at a 12°C incubation temperature show that the presence of chlorine is not enough to inhibit bacterial growth of the higher inoculum tested (104) (Table 3).

Table 1. Survival capacity of *Arcobacter butzleri* through time in different water matrixes incubated at 0°C.

Day	Chlorinated water		Non-chlorinated water		Non-chlorinated water + organic matter	
	10^2 inoculum	10^4 inoculum	10^2 inoculum	10^4 inoculum	10^2 inoculum	10^4 inoculum
1	-	-	+	+	+	+
3	-	-	-	+	+	+
5	-	-	-	-	-	-
7	-	-	-	-	-	-
9	-	-	-	-	-	-
11	-	-	-	-	-	-
13	-	-	-	-	-	-
15	-	-	-	-	-	-

+ Growth; - No growth.

Table 2. Survival capacity of Arcobacter butzleri through time in different water matrixes incubated at 5°C.

Day	Chlorinated water		Non-chlorinated water		Non-chlorinated water + organic matter	
	10^2 inoculum	10^4 inoculum	10^2 inoculum	10^4 inoculum	10^2 inoculum	10^4 inoculum
1	-	-	+	+	+	+
3	-	-	-	+	+	+
5	-	-	-	-	-	-
7	-	-	-	-	-	-
9	-	-	-	-	-	-
11	-	-	-	-	-	-
13	-	-	-	-	-	-
15	-	-	-	-	-	-

+ Growth; - No growth.

Table 3. Survival capacity of *Arcobacter butzleri* through time in different water matrixes incubated at 12°C.

Day	Chlorinated water		Non-chlorinated water		Non-chlorinated water + organic matter	
	10^2 inoculum	10^4 inoculum	10^2 inoculum	10^4 inoculum	10^2 inoculum	10^4 inoculum
1	-	+	+	+	+	+
3	-	+	+	+	+	+
5	-	+	-	+	+	+
7	-	+	-	+	-	+
9	-	+	-	+	-	+
11	-	-	-	+	-	-
13	-	-	-	-	-	-
15	-	-	-	-	-	-

+ Growth; - No growth.

For non-chlorinated samples, low inoculum survives for no more than 3 days and 5 days if organic matter is added, and for high inoculum, survival lasts for 9 days and 11 days in the presence of additional organic matter.

At Table 4 it is shown that Arcobacter survives in all the matrixes tested and for more than 15 days when the incubation temperature is of 25°C.

Table 4. Survival capacity of Arcobacter butzleri through time in different water matrixes incubated at 25°C.

Day	Chlorinated water		Non-chlorinated water		Non-chlorinated water + organic matter	
	10^2 inoculum	10^4 inoculum	10^2 inoculum	10^4 inoculum	10^2 inoculum	10^4 inoculum
1	+	+	+	+	+	+
3	+	+	+	+	+	+
5	+	+	+	+	+	+
7	+	+	+	+	+	+
9	+	+	+	+	+	+
11	+	+	+	+	+	+
13	+	+	+	+	+	+
15	+	+	+	+	+	+

+ Growth; - No growth.

Statistical analysis of the results obtained show, with a 95% significance, that the amount of CFU/mL inoculated in water correlates with the number of bacteria that can grow on it, and that incubation temperature has a significant effect over the bacterial survival. Nevertheless, the different matrixes evaluated show no statistical difference on the survival of this bacteria.

4. Discussion

In the last years, *Arcobacter butzleri* has been recognized as an emergent food borne pathogen and as mentioned above, water and animal origin food are the most probable sources of infection (Girbau et al. 2017). This bacterium has been associated with some waterborne outbreaks, where failures with the chlorinating process used for disinfection of drinking water have been considered as principal cause (Assanta et al. 2002).

Results obtained show that this bacterium can grow in all the water matrixes evaluated, regardless of the presence or not of residual disinfecting agent. Nevertheless, survival is greater in the non-chlorinated matrixes (with and without added organic material) than in chlorinated matrix. Similar findings have also been described by different authors, which concluded that organic material supplies protection to the bacteria especially at low incubation temperatures (Giacometti et al. 2015; Girbau et al. 2017).

Results obtained show that the survival of *Arcobacter butzleri* through time is directly proportional to incubation temperatures, being reduced as it decreases. The survival of this bacterium at low temperatures has been reported by several researchers, including Badilla-Ramírez et al. (2016), that demonstrated its survival at refrigeration temperatures or Kjeldgaard et al. (2009) that report bacterial growth and proliferation at 10°C.

With regard to water matrixes, Rice et al. (1999) have demonstrated the survival of *Arcobacter* in well water stored at 5°C for up to 16 days and Moreno et al. (2004) described the reduction in number only after 21 days of incubation in a water matrix at 12°C.

In natural conditions, free living amoeba including *Acanthamoeba castellanii* may contribute to the survival of the bacteria as temperature drops, establishing endocytobiosis that allows its survival for at least 10 days, as described by Villanueva et al. (2016).

Results obtained also show that the survival of *Arcobacter butzleri* decreases in all matrixes tested as time increases, except for incubation temperature of 25°C that is their optimal growth temperature. This might be due to the consumption of nutrients and the generation and accumulation of metabolites that lead to bacterial death (Kjeldgaard et al. 2009).

The use of chlorinated water should offer safety for the consumers avoiding bacterial contamination. Nevertheless, Shah et al. (2012) described the isolation of *Arcobacter butzleri* in 3% and 11.1% of potable

water samples in Turkey and Malaysia respectively. The isolation of this bacterium from potable water treatment plants might be explained based on an inadequate water chlorination procedure or the ability of this microorganism to form biofilm, generating a protective environment over potabilization processes (Assanta et al. 2002). Biofilm formation is influenced by several conditions including bacterial density, aerobic atmosphere and even incubation temperature (Fernandez et al. 2008; Kjeldgaard et al. 2009; Girbau et al. 2017).

5. Conclusion

This bacterium survives in all the water matrixes evaluated, regardless of the presence or not of residual disinfecting agent and, coinciding with the hypothesis, survival was greater in the non-chlorinated matrixes than in chlorinated matrix. Thus, it could be inferred that the transmission of *Arcobacter butzleri* by both, non-potable and drinking water is possible and might be one of the main sources of infection. Further research shall be done to better understand the behavior of this microorganism, in order to generate preventive measures that could decrease public health risk.

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