

Title: SURVIVAL OF ARCOBACTER BUTZLERI IN ENDOSYMBIOSIS WITH ACANTHAMOEBA POLYPHAGA, A. LENTICULATA AND A. RHYSODES

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Abstract:

Arcobacter butzleri is a potential zoonotic agent, with animal production and clinical significance, currently considered an emerging enteropathogen. It has been isolated from different sources like environmental waters, suggesting that it could share this ecological niche with other organism, such as free living amoebas (FLA) like, *Acanthamoeba polyphaga*, *A. lenticulata* and *A. rhysodes*. In order to characterize the endosymbiotic relationship between these microorganisms, the aim of this research was to determine the survival of *A. butzleri* inside these three FLA species. The survival of the field *A. butzleri* strain at different times of infection (1 – 2 – 5 - 10 – 15 – 20 – 25 – 30 days) was evaluated having as a negative control a pure bacterial culture. To conduct the survival assays, the FLA concentration was adjusted to 6×10^4 amoebae/mL in 1 mL of buffer PAGE and then inoculated with *A. butzleri* at MOI 100 and incubated at 27 °C in aerobiosis. When each time of infection was completed, the cultures were incubated for one hour with 20 µg/mL of gentamicin to eliminate extracellular bacteria. Then, washed three times with buffer PAGE and in the last wash, 250 µL were used for extracellular bacteria count. To each remaining pellet, 100 µL of sodium deoxycholate at 0.5% were added, incubating for 30min at room temperature to destroy the trophozoite and release intracellular bacteria. After that, 250 µL was extracted to determine the viable bacteria count. Both counts were made by the 6x6 dilution method. The final tally was calculated in this way: N° of intracellular bacteria/mL = intracellular count – extracellular count. Assays were performed in duplicate. The results allowed to establish that *A. butzleri* is able to survive as an endosymbiont for 20 days into amoebic vacuoles of *A. rhysodes* and 25 days into *A. polyphaga* and *A. lenticulata*. The FLA maintained their vegetative state, indicating that the bacteria resist and avoid the digestion by FLA. This fact allowed inferring that FLA could be a link not described yet in the chain of transmission of *A. butzleri*, what could have a direct impact on the epidemiology of this bacteria.

Keywords: *Arcobacter butzleri*, *Acanthamoeba*, endosimbiosis.

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