

Title: ARCOBACTER BUTZLERI AND A. CRYAEROPHILUS CHANGE THEIR ADHESIVE AND INVASION CAPACITIES TO HEP-2 CELLS AFTER SUCCESSIVE PASSAGES THROUGH ACANTHAMOEBA CASTELLANII TROPHOZOITES

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Abstract:

Arcobacter butzleri and *A. cryaerophilus* are potential enteropathogens and zoonotic agents involved in bacteremia and diarrheas in human beings. They have been isolated from environmental waters sharing this environment with other human pathogens, such as *Acanthamoeba castellanii*, a ubiquitous protozoa widely distributed in nature. The amoeba feed on bacteria, however, some bacterial species can resist amoebic digestion and remain intact within vacuoles allowing them to withstand adverse conditions and probably enhancing their virulence factors. The aim of this study was to determine variations in adhesion and invasion abilities of *A. butzleri* and *A. cryaerophilus* after 5 successive passages through *A. castellanii*. Axenic cultures of *A. castellanii* were infected with the bacterial species and the co-cultures were incubated at 26 °C for 4h (infection period). Then, the co-cultures were incubated for one hour with 60 µg/mL of gentamicin (elimination of extracellular bacteria) and washed with PBS for removing gentamicin. After 7 days, an aliquot of the co-cultures was plated on blood agar and incubated at 30 °C for 24-48h in aerobiosis to confirming absence of extracellular bacteria. Amoebas were lysed with 100 µL of sodium deoxycholate at 0.5%. To recover intracellular bacteria, 500 µL of the lysate was plated on blood agar and incubated at 30 °C for 24h in aerobiosis (this process corresponds to 1 of 5 passages). With the colonies obtained, were made the following passages and adherence and invasion assays in Hep-2 cells were performed inoculating cell monolayers with 1 mL of the bacterial suspensions (at 2 McFarland). The percentage change in adhesion/invasion compared to the native strain not passed through the amoeba (control) was determined. The significance of the data was analyzed by unpaired t-test $\alpha=0.05$, GraphPad Prism. Assays were performed in duplicate. The results indicate that after 5 successive passages in *A. castellanii*, both bacterial species increased their adhesion and invasion ability at least in 50 and 60% respectively. This suggest that *A. butzleri* and *A. cryaerophilus* in the transient permanence inside *A. castellanii* trophozoites enhance their capacity to adhere and invade Hep-2 cells, becoming more virulent.

Keywords: *A. castellanii*, *A. butzleri*, *A. cryaerophilus*, virulence, adhesion, invasion.

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