

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024

Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.



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ADAPTATION STRATEGIES OF *Burkholderia contaminans* TO SURVIVE UNDER LONG-TERM STARVATION CONDITIONS

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Burkholderia contaminans, one of the 24 members of the *Burkholderia cepacia* complex, is an opportunistic pathogen often recovered from patients with cystic fibrosis and immunocompromised individuals. It is widely distributed in natural environments such as plants, water, and soil. Due to its high genetic plasticity, it can remain viable for extended periods in hostile environments¹. It has been isolated from nutrient-poor solutions, pharmaceutical products like nasal sprays, disinfectants, and medical devices; however, the mechanisms behind its persistence remain largely unexplored. This study aims to unravel the phenotypic strategies that *B. contaminans* uses to survive under long-term nutrient limitation. In this study, we used the *Burkholderia contaminans* CAMPA 320 ST 482 isolate recovered from a contaminated water tank in a hemodialysis unit of a local hospital. A 16-hour LB culture of this isolate was washed thoroughly and used to inoculate 1 L of sterile water (10^8 CFU/mL). The flask was incubated at 37°C for 1 month, with four biological replicates. This culture was monitored at 0, 24, and 48 hours, 1, 2, 3, and 4 weeks. At each time point, bacterial survival rates were quantified by CFU/mL, and cell size and morphology were assessed by light microscopy. Cell membrane permeability was evaluated through crystal violet staining. Live-dead bacteria and changes in cellular aggregation over the incubation time were assessed using fluorescence microscopy (Syto9-IP staining). Cell integrity and cytoplasmic contents were analyzed using Transmission Electron Microscopy. Changes in cell lipid composition, protein secondary structure, and the dynamics of the synthesis and utilization of intracellular storage materials were analyzed by infrared spectroscopy (IR).

Our results show that *B. contaminans* retained cell viability throughout the entire incubation period, oscillating from 10^8 to 10^6 CFU/mL along the culture. We observed a reduction in cell size and decreased membrane permeability. Significant changes in internal cell structure, such as contraction/depletion of cytoplasmic content, were noted. Bacterial aggregates formed after 24 hours, and their structure, size, and composition evolved to be more complex after two weeks of starvation. Additionally, IR studies revealed significant changes in protein conformation and marked modifications in cell lipid composition after 2 and 3 weeks of incubation, respectively.

These adaptations underscore the high complex strategies employed by *B. contaminans* to enhance its survival and resilience under prolonged starvation conditions. It is concerning that one of the most significant consequences of these adaptive mechanisms is the structural and biochemical changes in the cell envelope, which may contribute to increased antimicrobial tolerance. This highlights the serious risks posed by *B. contaminans* contamination in hospital environments.

Palabras clave: *Burkholderia contaminans* - Starvation - Adaptative response - Contamination - Hemodialysis unities