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Abstract (oral session)

Glutamate racemase is a critical gene for Acinetobacter baumannii biofilm formation and attachment to eukaryotic cells

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Objectives: The ability of A. baumannii to form biofilms on abiotic surfaces and adhere to eukaryotic cells is central to its survival success and pathogenicity. We found that the metabolism of L-glutamate is essential for biofilm formation (Cabral el at., 2011), as well as the presence of the D-glutamate isomer. Glutamate racemase is one of the two enzymes that catalyses the formation of D-glutamate, which is necessary for cell wall peptidoglycan synthesis. We examined the roles of the two glutamate racemase genes of A. baumannii, named murl1 and murl2, during abiotic biofilm formation and interaction with HeLa cells. Methods: The murl1 and murl2 mutants of A. baumannii ATCC 17978 were obtained by disruption of the target genes by single homologous recombination events with internal fragments of these genes cloned into the pCR-Blunt II-TOPO vector. Abiotic biofilms were cultivated in 96-well polystyrene plates, stained with crystal violet and quantified at 570 nm. HeLa cells were grown in six-well plates or coverslips placed in six-well plates. Confluent monolayers were infected with 2 x 10^6 bacterial cells, and incubated for 1 h at 37ºC. After washing, the infected monolayers were lysed and lysate dilutions were plated to determine the number of bacteria attached to HeLa cells. For SEM analysis, the coverslips were fixed to aluminium stubs, coated with gold and examined with a Jeol JSM-6400 scanning electron microscope. Results: The murl1 and murl2 mutants did not form biofilms on polystyrene when L-glutamate was provided as the only carbon source. In the presence of another source of carbon, the murl1 mutant still produced no visible biofilm, while murl2 showed similar levels to the parental strain. The murl1 mutation was found to drastically impair biofilm formation at 48, 72 and 96 h of incubation. Both murl1 and murl2 mutations affected bacterial attachment to HeLa cells, with no viable counts obtained after eukaryotic cell lysis. SEM showed almost no attachment of murl1 mutant to eukaryotic cells, when compared with the parental strain (see Figure 1). Conclusion: L-D Glutamate interconversion is a key process for A. baumannii to form abiotic biofilms and attach to HeLa eukaryotic cells. Thus, the Glutamate racemase murl1 gene could ultimately provide and effective target for controlling biofilms and pathogenicity.

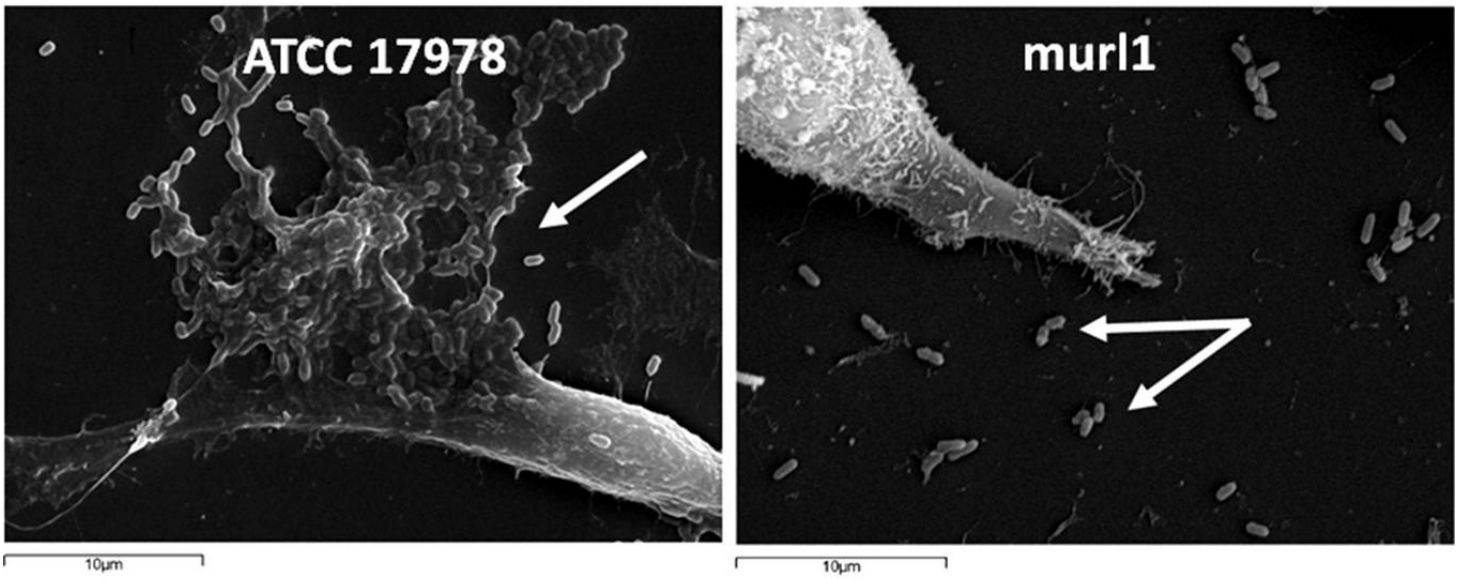

Figure 1. SEM analysis of bacterial attachment to eukaryotic cells. Confluent monolayers of HeLa cells were incubated with 2 x 10^6 cells of *A. baumannii* ATCC 17978 parental strain (left panel) or murl1 mutant strain (right panel). The arrows indicate bacterial cells.