Manipulating The PAAR Proteins of Acinetobacter Baumannii

Authors : Irene Alevizos, Jessica Lewis, Marina Harper, John Boyce

Abstract: Acinetobacter baumannii causes a range of severe nosocomial-acquired infections, and many strains are multi-drug resistant. A. baumannii possesses survival mechanisms allowing it to thrive in competitive polymicrobial environments, including a Type VI Secretion System (T6SS) that injects effector proteins into other bacteria to give a competitive advantage. The effects of T6SS firing are broad and depend entirely on the effector that is delivered. Effects can include toxicity against prokaryotic or eukaryotic cells and the acquisition of essential nutrients. The T6SS of some species can deliver 'specialised effectors' that are fused directly to T6SS components, such as PAAR proteins. PAAR proteins are predicted to form the piercing tip of the T6SS and are essential for T6SS function. Although no specialised effectors have been identified in A. baumannii, many strains encode multiple PAAR proteins. Analysis of PAAR proteins across the species identified 12 families of PAAR proteins with distinct C-terminal extensions. A. baumannii AB307-0294 encodes two PAAR proteins, one of which has a Cterminal extension. Mutation of one or both of the PAAR-encoding genes in this strain showed that expression of either PAAR protein was sufficient for T6SS function. We employed a heterologous expression approach and determined that PAAR proteins from different A. baumannii strains, as well as the closely related A. baylyi species, could complement the A. baumannii Δpaar mutant and restore T6SS function. Furthermore, we showed that PAAR fusions could be used to deliver artificially cloned protein fragments by generating Histidine- and Streptavidin- tagged PAAR specialised effectors, which restored T6SS activity. This provides evidence that the fusion of protein fragments onto PAAR proteins in A. baumannii is compatible with a functional T6SS. Successful delivery by this mechanism extends the scope of what the T6SS can deliver, including user designed proteins. Keywords : A. baumannii, effectors, PAAR, T6SS

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1