TAGGING *A. BAUMANNII* TYPE VI SECRETION SYSTEM COMPONENTS WITH A GREEN FLUORESCENT PROTEIN

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Acinetobacter baumannii is a Gram-negative opportunistic pathogen responsible for hospital-acquired nosocomial infections [1], [2]. It is a successful pathogen due to its ability to resist desiccation, disinfectants and major antimicrobials [3]. Previously identified *A. baumannii* two-component signal transduction system BfmRS was shown to be responsible for regulating virulence-related traits such as biofilm production, resistance to antibiotics, type VI secretion system (T6SS) regulation, survival in human ascites fluid and serum [4]. T6SS is also related to *A. baumannii* virulence and responsible for inter-bacterial competition and bacterial interactions with eukaryotic cells [5]. Secretion systems are usually regulated by two-component signal transduction systems. However, T6SS regulation and BfmRS role in it are not fully understood. Therefore, in this work, we aimed to fluorescently label T6SS components in *A. baumannii*.

Markerless gene deletion technique was used to generate Δ*bfmRS*, Δ*hcp*, and Δ*bfmRS*Δ*hcp* mutants. The total protein content of mutants was visualized using SDS-PAGE. The inter-bacterial competition assay was performed by incubating mixed bacterial strains at the aggressor (*A. baumannii*) and prey (*E. coli*) ratio 10:1, respectively. Components of T6SS (Hcp and TssB) were fused with a green fluorescent protein by the PCR-based overlap extension method. Labeled proteins were tracked by a fluorescent microscope with a 600x-1000x magnification range.

Protein secretion profiles of *A. baumannii* clinical strain and its Δ*bfmRS* mutant revealed that the mutant displayed a reduction of Hcp protein, which is essential for the assembly of the T6SS apparatus. However, competition assays showed that loss of bfmRS did not impair the killing phenotype. To evaluate the assembly state of T6SS in the mutant, Hcp protein was fluorescently labeled with a green fluorescent protein in N- and C- termini. However, fluorescent labels *per se* impaired killing phenotype and Hcp tracking did not reveal any information about the state of T6SS. Therefore, we then fluorescently labeled sheath protein TssB and evaluated T6SS activity.

Fluorescently labeled T6SS with Hcp protein was non-functional. However, T6SS component TssB looks like a promising candidate to track T6SS in *A. baumannii*.

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