Calcium Deposition as a Novel Virulence Factor in *Pseudomonas aeruginosa*

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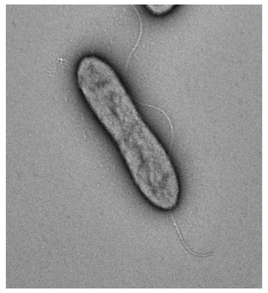
Abstract

Calcium (Ca) deposition in soft tissues has been associated with several bacterial infections including cystic fibrosis (CF). In CF patients, the human pathogen *Pseudomonas aeruginosa* is the predominant cause of death. We identified three β-class CAs, psCA1, psCA2 and psCA3 in *P. aeruginosa.* Currently, we aim to determine the role of psCA2 and psCA3 in the virulence. We are also testing the effect of various CA inhibitors on the ability of *P. aeruginosa* to infect and kill *Galleria*. To study the regulation of psCA genes, we have cloned the promoter regions of the corresponding genes upstream of the *lux* operon. This will enable monitoring the transcriptional activation of the genes in response to various host factors, including elevated Ca. These results will provide insights into the role of CAs in *P. aeruginosa* virulence and assist the efforts of developing new treatments for infected patients.

Pseudomonas aeruginosa



- A deadly human pathogen that causes nosocomial infections mostly in immunocompromised patients.
- Causes acute and chronic infections including pneumonia, bacteremia and wound infections, ear and eye infections and urinary tract infections.
- Major cause of death in patients with Cystic Fibrosis (CF), a genetic condition causing the mucus in the lungs to become thick and sticky.
- Highly antibiotic-resistant, with WHO ranking it as the second most dangerous pathogen in terms of antibiotic resistance.

Soft tissue Calcification, Calcium deposition and human diseases

- The Ca^{2+} levels in human body tissues and fluids are tightly regulated.
- Abnormally elevated Ca^{2+} levels are associated with diseases, including CF, cardiovascular disease and arteriosclerosis.
- Such elevated levels of Ca^{2+} levels may lead to soft tissue calcification and deposition of Ca^{2+}
- Calcification may involve formation of calcium carbonate ($CaCO_3$) deposits.
- $CaCO_3$ deposition may rely on activities of **carbonic anhydrases (CA)** which reversibly hydrate carbon dioxide (CO_2)

$$H_2O + CO_2 \xrightleftharpoons{CA} H^+ + HCO_3^-$$
$$Ca + HCO_3^- \rightleftharpoons CaCO_3 + H^+$$

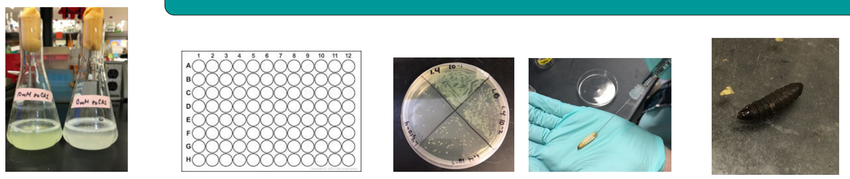
- Calcium deposition is also observed during bacterial infections, such as tuberculosis and infective endocarditis.

Hypothesis :

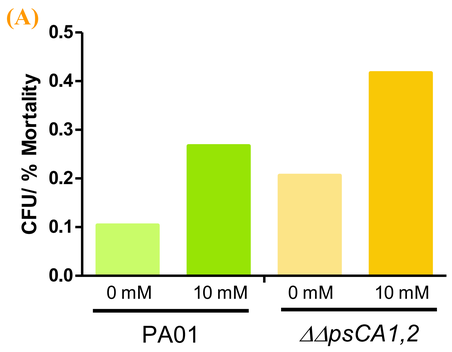
➢ *Pseudomonas aeruginosa* is capable of $CaCO_3$ deposition, which enhances its virulence and pathogenicity.

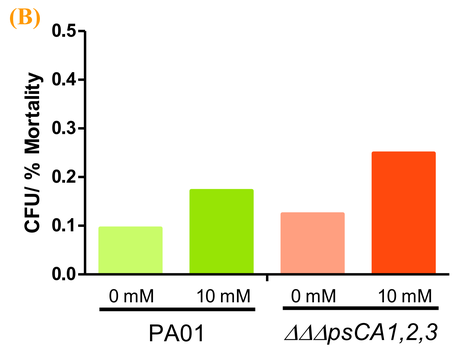
➢ $CaCO_3$ deposition by *P. aeruginosa* requires at least one of three beta-carbonic anhydrases encoded by the organism.

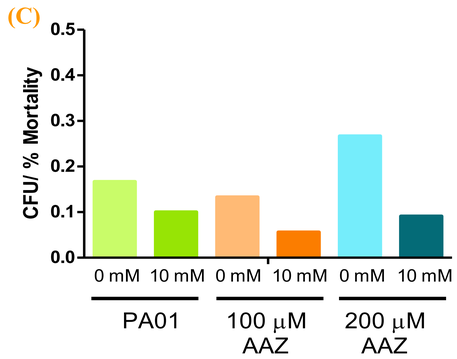
Galleria mellonella infection model



PA01 & mutant cultures were grown at 0 mM or 10 mM $CaCl_2$ in BMM → Cultures were normalized, then diluted to achieve the desired infectious dose. → The infectious doses were verified by plating and CFU count, Worms were then injected. → Worms were incubated at 37°C and checked for death over a 24 hour period → Data was analyzed and used to build killing curves and mortality graphs

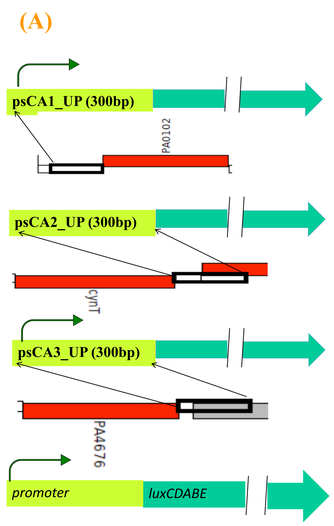


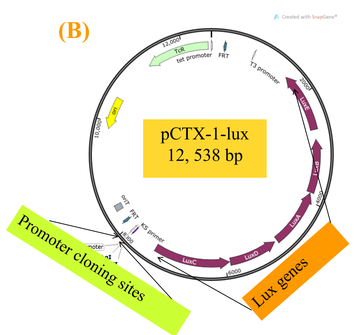


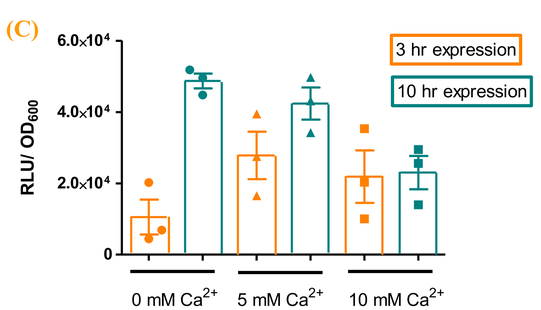


(A) The WT (PA01) was grown along side a double deletion mutant lacking psCA1 and psCA2 genes, Δ*psCA1,2,* at 0 mM calcium and 10 mM calcium. This graph shows the normalized data of the colony forming units (CFU) when compared to the percent mortality seen in the worms injected with these conditions. As you can see there is a clear calcium impact on the killing of *G. mellonella.*
(B) For this experiment the WT alongside a triple deletion mutant lacking all three of the carbonic anhydrases found in *P. aeruginosa*, Δ*psCA1,2,3*. Looking at the graph you can still see a calcium impact, although it is less than that seen in graph A.
(C) This graph shows the WT ran in three different conditions: no inhibition, in the presence of 100 µM acetazolamide (AAZ), and in the presence of 200 µM AAZ. In this graph we see an inversion with the impact showing in conditions grown in the absence of calcium.

Ca^{2+} regulates the expression of psCAs in *P. aeruginosa*







(A),(B) To determine the effect of higher levels of Ca^{2+} (10mM) on the transcription of *psCA*s in *P. aeruginosa*, promoter fusions were constructed by cloning 300 bp regions upstream of each of the CA genes upstream of promoterless *luxCDABE* operon in the pCTX-1-lux vector. The constructs were transformed by mating into PAO1, generating PAREB1, PAREB2 and PAREB3 strains.
(C) Promoter activity assay results showing PAREB1 at the beginning and end of the log phase. There you can see an early rise with the addition of calcium compared to no added calcium.