REVOLUTIONIZING BIOMEDICAL RESEARCH: INTEGRATING CUTTING-EDGE AI/ML TO UNLEASH INNOVATION IN DRUG DISCOVERY AND THERAPEUTICS DEVELOPMENT

Cell-penetrating Peptide Predictors To Design Delivery Vehicles For Morpholinos And Peptide-nucleic Acids To Pseudomonas Aeruginosa.

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Impact: The rise of multidrug-resistant bacteria, leading to increased infections in wounded warfighters, underscores the urgent need for innovative antimicrobial strategies.
Purpose: To combat this challenge, we discovered a novel Toxin-Antitoxin (TA) system in Pseudomonas (Pa) that could be modulated to induce bacterial cell death in antibiotic resistant bacteria, in the absence of traditional antibiotics.
Objective: We are characterizing this TA system for its expression and phenotypic effects on growth and biofilm.
Rationale: This new TA system consists of a putative pore-forming toxin gene and a potential cognate antitoxin gene that is proposed to repress toxin expression. We will deliver a peptide nucleic acid (PNA) molecule or morpholino complimentary to the antitoxin's mRNA to suppress antitoxin translation and lead to cell death from uncontrolled toxin expression using a cell penetrating peptide (CPP) approach. We designed these CPPs using Artificial Intelligence/Machine Learning (AI/ML) tools like CPPpred and CellPPD predictor.
MDR Pseudomonas forms stubborn biofilms, especially on medical implants and catheters, which are directly connected to disease outcomes in warfighters and diabetes patients. We have tested mutants in these TA genes and did bioinformatic modeling of the toxin/antitoxin proteins to understand their potential biochemistry. We have developed PNA/morpholinos to inhibit antitoxin expression. If successful, this work could lead to a new antimicrobial approach for MDR Pseudomonas infection. It may have broad-spectrum applications to killing other MDR bacteria as well.
Methods: AI /ML tools used to design CPPs. Bioinformatics programs used to predict structure of toxin protein as a homo-oligomer. Biofilm and growth assays of transposon insertion mutants in the putative TA genes as well as in a second related TA system.
Results: Analysis of the protein sequence was done to predict the oligomerization state of the proposed toxin protein and suggests a 7-mer protein complex, similar to many other bacterial toxins. Transposon insertion mutants in the TA genes were assessed for phenotypic differences, including biofilm formation and growth. One toxin mutant and one antitoxin mutant had 77% and 62% decreased biofilm formation in comparison to other mutants and wild type Pseudomonas. We observed differences between the crystal violet staining results and the colony biofilm assay: one method stains attached biofilm as opposed to pellicle biofilm. The role of the TA system in these two different biofilm compartments will be studied further.
Conclusion: This novel TA system was identified in Pseudomonas aeruginosa. The phenotypic effect of mutants in this TA system was found to include significant changes in biofilm formation with some mutants in TA genes, and no effect of mutants in the second TA system. A similar system has also been identified in other bacteria, suggesting broader applicability of this system to other potential human pathogens.

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