An RK2 Mediated Bacterial Conjugation Delivery System for Artificial Genes Coding for Antimicrobial Polypeptides: A Novel Synthetic Biology Approach to Antibiotic Resistance

Collins, Logan

Traditional antibiotics are becoming obsolete due to the growing danger of antibiotic resistance and their frequent toxicity to human indigenous microbiota. This investigation develops a synthetic biology treatment that approaches antibiotic resistance through the use of a novel artificial gene and bacterial conjugation. The treatment has the potential to be more dynamic, adaptable, and to cause less harm to indigenous microbiota than traditional antibiotics. The artificial gene disrupted bacterial systems by producing hydrophobic polypeptides. The broad host range conjugative plasmid RK2 was used to mobilize a pET11a plasmid carrying both an artificially synthesized RK2 origin of transfer (OriT) and the artificial gene controlled by a model recipient specific promoter. This allowed for the transfer of the antimicrobial producing artificial gene from non-sensitive donors to susceptible recipients. It was experimentally verified that the RK2 and the pET11aOriT plasmids could be transferred by conjugation. To demonstrate the toxicity of the artificial genes delivered by conjugation, donor E. coli c600 containing both RK2 and pET11aOriT plasmids were mated with recipient E. coli BL21 (DE3) which carried the GFP expressing pHL662 plasmid. A plate reader was used to measure the GFP fluorescence of the mating cultures. The growth of the recipient bacteria was significantly reduced ($p = 0.003$). If used to treat infections, this technique could be modified to target desired pathogens by selecting pathogen specific promoters to control the expression of T7 RNA polymerase. The donors could be delivered to the site of an infection and used to kill antibiotic resistant bacteria.

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