Production of Antimicrobial Peptides in Nicotiana benthamiana Plants for Efficient Treatments

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Antibiotic-resistant infections are projected to cause over 39 million global deaths between 2025 and 2050, with treatment costs exceeding $66 billion annually. Rather than replacing ineffective antibiotics, it is possible to restore them. Plant-produced antimicrobial peptides (AMPs) offer a promising yet untapped synergistic treatment. However, the absence of critical pre-clinical evaluations has hindered AMP's clinical translation. This study aims to express AMPs in Nicotiana benthamiana using a tobacco mosaic virus (TMV) vector for evaluation of purity, antibacterial, cytotoxicity, and synergistic properties. A TMV-AMP plasmid was inserted into agrobacteria for agroinfiltration into N. benthamiana. After TMV-AMP plant infection, AMPs were purified via RP-HPLC. AMP viability was assessed via PCR, an antibacterial assay on E. coli MG1655, a cytotoxicity assay on human embryonic kidney (HEK) and human dermal fibroblast (HDF) cells, and a synergy assay combining AMPs with azithromycin against resistant E. coli PI-7. AMPs exhibited antibacterial activity against E. coli MG1655, with a minimum inhibitory concentration of 50 µg/mL, achieving 92.46% ± 4.53 bacterial inhibition. AMPs showed minimal cytotoxicity to HEK and HDF, maintaining >90% cell viability up to 50 µg/mL. The optimal AMP concentration for minimal cytotoxicity and maximal antibacterial activity was 50 µg/mL, with a final plant-produced yield of 2.1 mg/mL, the highest reported for plant-TMV expression. AMPs enhanced azithromycin's efficacy against E. coli PI-7; a combination costing less than 1 cent per dose. This study highlights the potential of clinically translating AMPs as a cost-effective and clinically viable treatment to restore antibiotic efficacy, potentially saving lives by 2050.

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