Molecular basis of the mechanism of action of insect antimicrobial peptides

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INTRODUCTION

Antimicrobial resistance (AMR) is a pressing global health crisis, contributing to ~4.71 million deaths globally in 2021[1]. The ESKAPE pathogens evade traditional antibiotics, making the discovery of novel therapeutic agents critical[2]. Insect Antimicrobial Peptides (AMPs) offer a promising alternative, providing broad-spectrum defense by targeting fundamental bacterial structures, minimizing resistance evolution[3]. Our lab has identified putative AMPs from *Hermetia illucens* (Black Soldier Fly)[4]. The main objetive is to investigating whether these identified lead peptides utilize rapid membrane disruption (lytic) or complex, non-lytic intracellular mechanisms.

METHODS

This study employs a comprehensive production and characterization strategy. Our pipeline begins with cloning the lead sequence into a TA vector intermediate. Currently, these sequences are being subcloned into pPIC9K vectors to facilitate future heterologous expression in *Pichia pastoris* yeast.

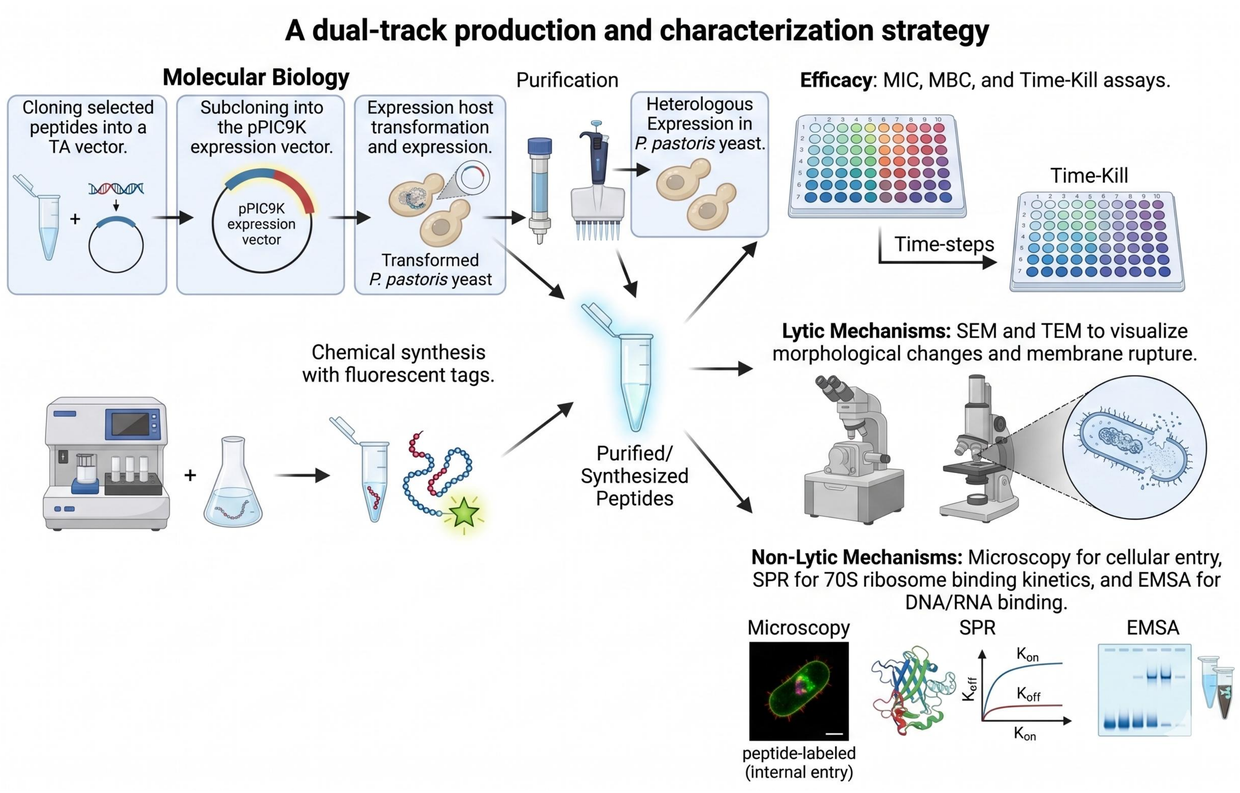

***Figure 1*: Laboratory workflow involving recombinant expression in *P. pastoris* and advanced microscopy techniques.**

In parallel, we will utilize chemical synthesis to generate analogous peptides incorporating tags. Once production and purification are complete, antimicrobial efficacy will be established by determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and lethality through time-kill assays. To determine the mechanism of action, we will use scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to check for bacterial membrane rupture. we will also investigate intracellular targets by confirming cellular entry via microscopy, measuring 70S ribosome binding kinetics with surface plasmon resonance (SPR), and evaluating direct DNA/RNA binding using electrophoretic mobility shift assays (EMSA).

RESULTS

We are successfully establishing a robust, reproducible pipeline for the production and characterization of BSF-derived peptides. The target peptide (C16162) sequence was successfully cloned into a TA vector and sequence-confirmed. This gel shows the subsequent double digestion using EcoRI and NotI (Figure 2).

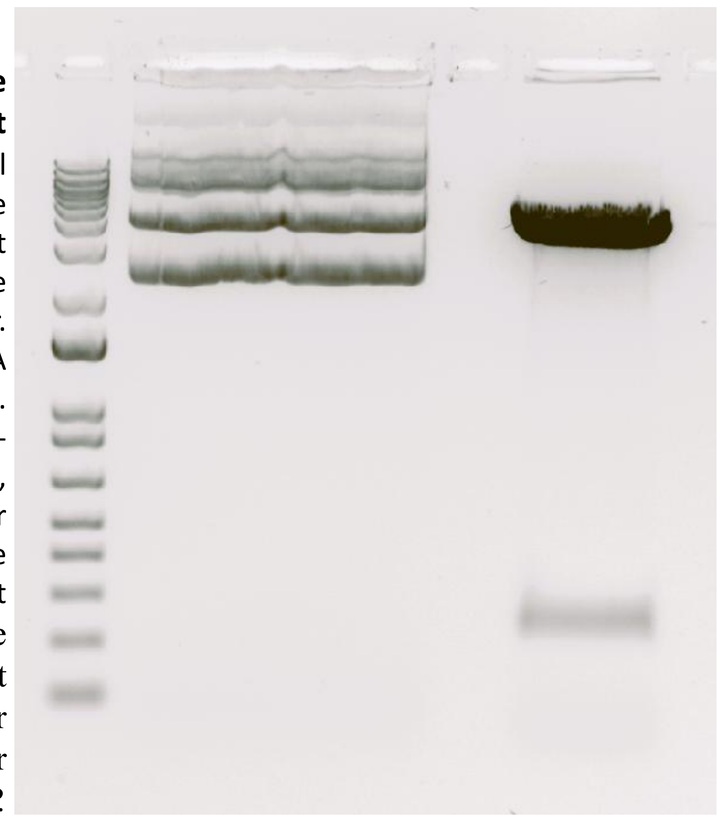

***Figure 2*: Preparation of the c16162 insert for yeast expression.** Agarose gel electrophoresis confirming the successful excision of the target sequence. Lane 1 contains the Invetrogen™ 1KB Plus DNA Ladder. Lane 2 shows the undigested TA vector containing the c16162 insert. Lane 3 shows the TA vector double-digested with EcoRI and NotI, resulting in the larger vector backbone (upper band) and the successfully excised C16162 insert (lower band at 218bp). The successful excision of the target insert confirms it is ready for ligation into the pPIC9K vector for heterologous expression in *P. pastoris*.

CONCLUSIONS

The successful cloning and preparation of target peptide C16162 establishes a validated, reproducible pipeline for generating BSF-derived AMPs. By transitioning this sequence into our yeast expression system, we are now positioned to produce the high-purity peptides needed to elucidate their specific lytic or non-lytic mechanisms[5]. Ultimately, defining these exact molecular pathways will accelerate the translation of insect-derived molecules into viable clinical therapies against the global AMR crisis.

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