Patti Territoriali
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INSECT-DERIVED ANTIMICROBIAL PEPTIDES: PRODUCTION, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY *IN VITRO* AND *IN VIVO*

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Introduction

Antimicrobial resistance is one of the major global public health challenges, with significant impacts on human and veterinary medicine as well as the global economy[1]. The widespread and often indiscriminate use of antibiotics has accelerated the emergence of multidrug-resistant strains, reducing the effectiveness of existing treatments and creating an urgent need for new therapeutic strategies[2]. Among the most promising alternatives are antimicrobial peptides (AMPs), naturally occurring molecules of the innate immune system that exhibit broad-spectrum antimicrobial activity[3]. A key advantage of AMPs is their low tendency to induce resistance, due to their rapid mechanism of action and targeting of essential microbial components. Insects represent a valuable source of AMPs because of their biodiversity and constant exposure to microbial threats[4]. In particular, the black soldier fly (*Hermetia illucens*) has gained attention as a model organism, both for its role in organic waste bioconversion and for its rich repertoire of antimicrobial peptides[5].

Methods

The work focuses on designing and constructing expression vectors for AMPs from *H. illucens*. Protocols for larval immune stimulation using Gram-positive and Gram-negative bacteria, as well as hemolymph extraction and peptide purification, were defined. In parallel, a recombinant production strategy was developed: target sequences were selected from literature and databases, codon-optimized, amplified, and cloned into TOPO vectors, then validated in *Escherichia coli* through screening and sequencing. Finally, suitable vectors and preliminary conditions for heterologous expression in *Pichia pastoris* were established, including initial optimization of culture and induction parameters (Fig. 1).

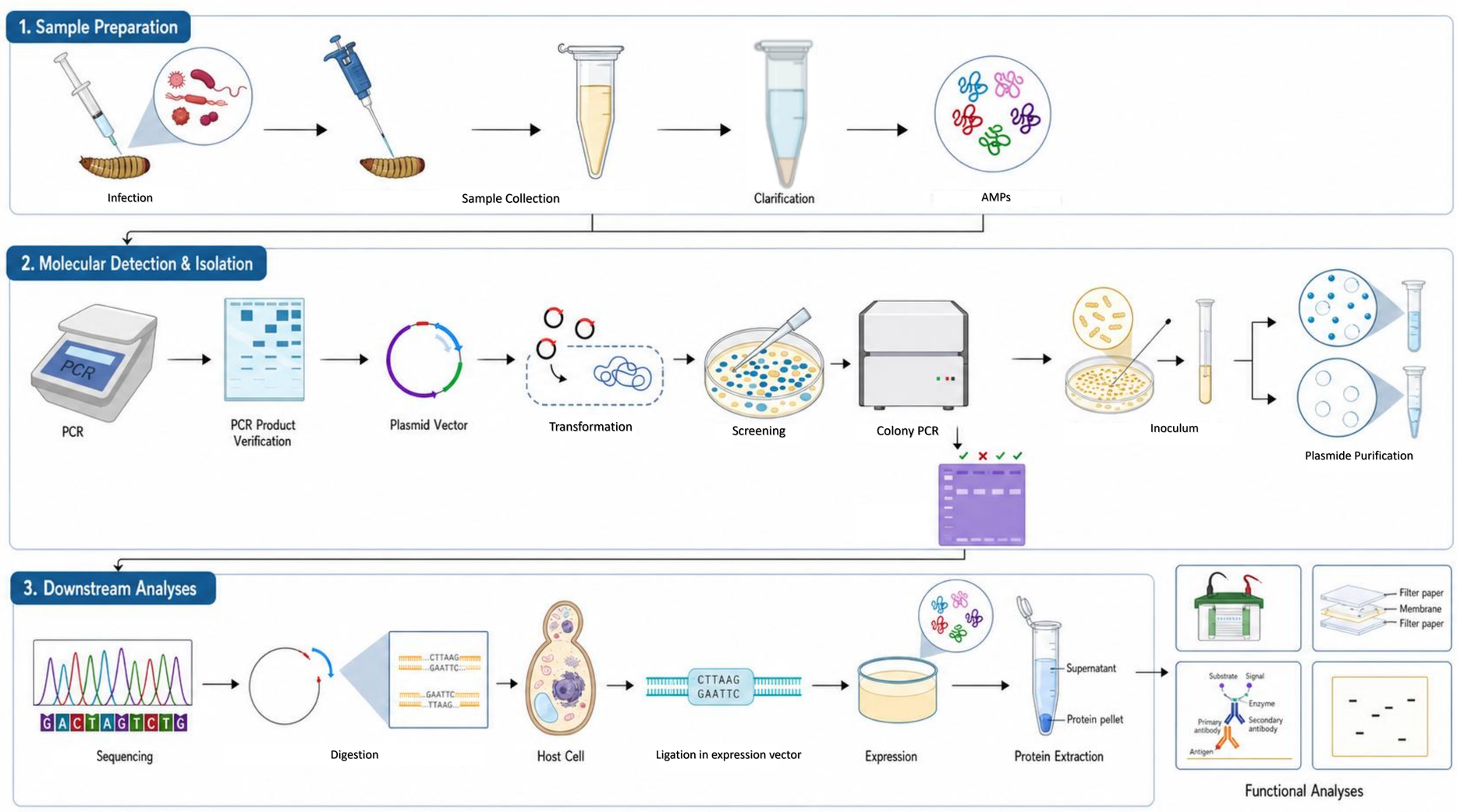

Figure 1. Schematic representation of the experimental pipeline for molecular and functional analysis. The process begins with Sample Preparation, involving the infection of *Hermetia illucens* larvae, followed by homogenization and clarification to isolate peptides. In the *Molecular Detection & Isolation* phase, the target sequences are amplified via PCR, cloned into plasmid vectors, and transformed into competent cells for screening and archiving. Finally, Downstream Analyses include sequencing for plasmid confirmation, protein expression in heterologous host cells, and final functional validation through Western Blotting and enzymatic assays.

Results

Target antimicrobial peptide sequences derived from *H. illucens* were identified and prioritized based on structural features and predicted biological activity. Genetic constructs were designed and an initial library of recombinant clones was obtained. Molecular analyses confirmed correct integration and sequence integrity, validating constructs for heterologous expression (Fig 2). In particular, the C7081 peptide was successfully transferred into the eukaryotic system *P. pastoris*, representing a first step toward recombinant production. Additional peptide candidates are currently under molecular evaluation for selection of the most promising constructs. A preliminary expression pipeline has been established, and initial culture and induction conditions have been defined.

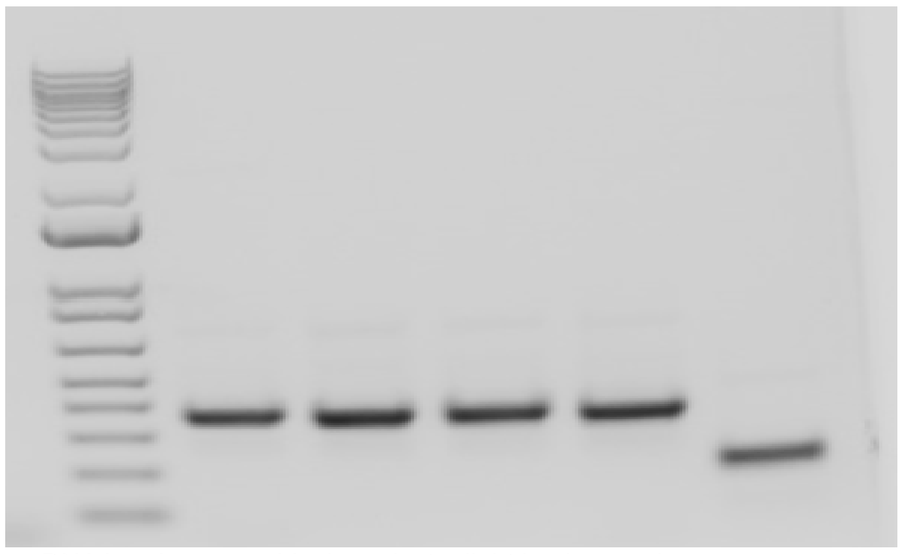

Figure 2. Colony PCR screening of *E. coli* transformants for peptide C7081 cloning. A representative agarose gel electrophoresis image shows the analysis of five independent colonies selected from a white/blue screening plate. The image is an inverted black and white scan where DNA bands appear black against a light background.
Lane 1: DNA Ladder; Lanes 2-4: PCR products from four white colonies (putative positive), showing a higher molecular weight band. This shift indicates the successful integration of the C7081 peptide sequence (recombinant clones); Lane 5: PCR product from a blue colony (putative negative), showing a faster-migrating band. This smaller fragment corresponds to the empty vector without the insert (non-recombinant clone).
The results confirm the efficiency of the white/blue screening, with lanes 2-4 identifying positive clones suitable for subsequent expression and characterization.

Conclusions

The study confirms the feasibility of identifying and engineering AMPs from *H. illucens* as promising tools against antimicrobial resistance. The successful construction and validation of recombinant vectors mark a key step toward alternative antimicrobial strategies, providing a solid basis for further work on peptide expression, purification, and characterization. The integration of immune stimulation in larvae and recombinant approaches strengthens the study by enabling exploration of both natural and engineered peptides. Future work will focus on optimizing heterologous expression systems, particularly in *P. pastoris*, and on functional assays to assess antimicrobial activity and cytotoxicity. Overall, this research lays the groundwork for developing innovative, sustainable AMP-based therapeutics.

References

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