Exploring Antimicrobial Potential of *Mytilus* Genomic Resources

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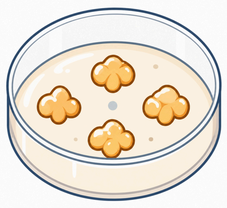
Abstract
The global healthcare community faces escalating threats from antimicrobial resistance (AMR); therefore, the need for novel, non-traditional antimicrobials has become increasingly urgent. Antimicrobial peptides (AMPs), naturally produced by a wide array of organisms, including marine invertebrates, offer a promising alternative to conventional antibiotics. AMPs typically act by disrupting microbial membranes, a mechanism less prone to resistance development. Mussels, being sessile filter feeders in pathogen-rich marine environments, have evolved expansive and diverse innate immune systems. The genomic diversity and adaptive resilience of *Mytilus* spp. remain underexploited resources for drug discovery, particularly in the search for new AMPs with clinical and ecological relevance.
The central objective of the project is to identify, functionally validate, and characterize novel AMPs from *Mytilus* genomes. The research plan integrates *in silico* genomic screening with experimental *in vitro* validation. Initially, genome assemblies and transcriptomic datasets from multiple *Mytilus* species will be analyzed to identify gene families coding for AMP-like sequences. The bioinformatics strategy includes sequence motif detection, gene clustering, evolutionary analysis of presence/absence variation, and structural modeling of predicted peptides. These tasks will be supported by national HPC resources provided through the PLGrid infrastructure, which allows high-throughput data processing, molecular simulations, and structural predictions. Experimentally, we will establish short-term primary cultures from *relevant Mytilus* cell types. These cultures will serve as models to assess immune gene expression and AMP induction under controlled stimulation. Culture viability and responsiveness will be monitored using fluorescein diacetate (FDA) assays and quantitative PCR. Peptides derived from highly expressed or computationally prioritized genes will be purified either from conditioned media or synthesized chemically when necessary. Functional testing will involve comprehensive antimicrobial profiling against a library of multidrug-resistant strains.

Primary cell cultures

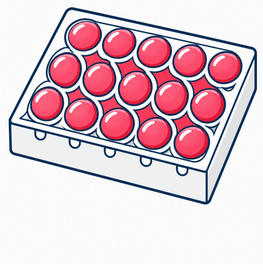
Mussel collection and tissue extraction



Tissue dissociation (enzymatic & mechanical)

Primary cell culture establishment

Viability manitoring (FDA staining)

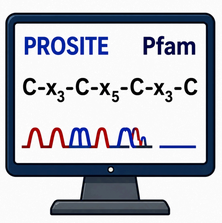


Media optimization (osmolarity, pH, antibiotics)

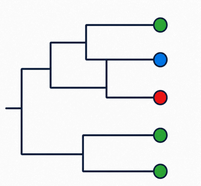
Reproducible cultures from several tissues for AMP expression experiments

Data mining

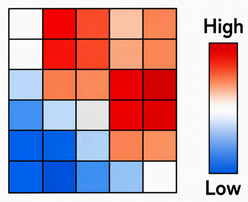
Mussel genomic & transcriptomicc datasets



Conserved motif search & signal peptide prediction



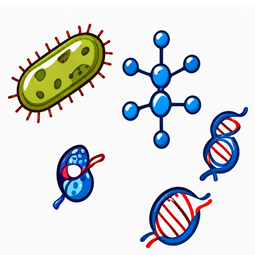
Gene family expression analysis (phylogenetic clustering)



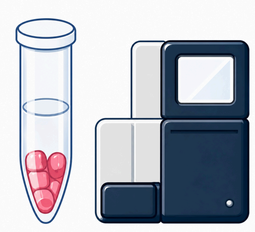
Expression potential (public & in-house RNA-Seq data)

Prioritized AMP gene candidates

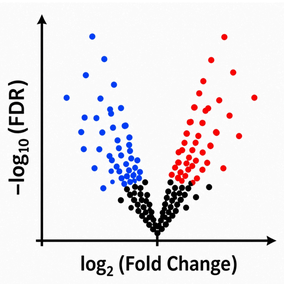
Induction and expression profiling



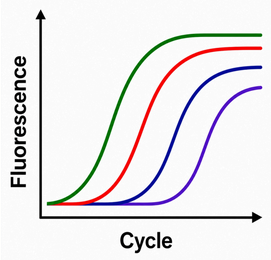
Immune stimulation (LPS, bacterial lysates, live AMR strains)



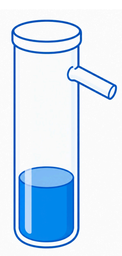
RNA extraction & RNA-Seq (full transcriptome profiling)



Differential expression analysis to identify induced AMP genes

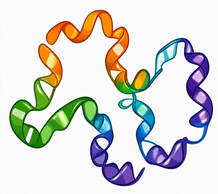


Validation of candidate AMP genes (RT-qPCR)

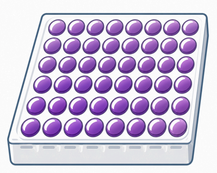


Prioritized AMPs & assessment of secretion into culture medium (HPLC fractionation)

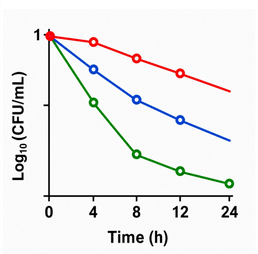
Peptide purification and functional testing



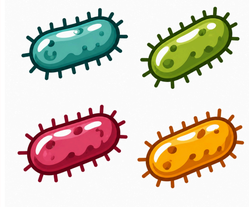
Peptide synthesis or purification from culture supernatants (HPLC/RP-HPLC)



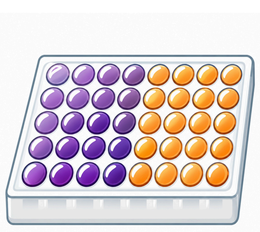
In vitro antimicrobial activity assessment (broth dilution, MIC assays)



Time-kill kinetics & bacterial activity



Sectrum of activity & resistance profiles against AMR strains



Synergy with antibiotics (checkerboard assays)

Lead AMPs with therapeutic potential

Outcome
Identification and validation of potent, broad-spectrum AMPs from mussel primary cells as novel leads against antibiotic-resistant bacterial pathogens.

National Science Centre Poland

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