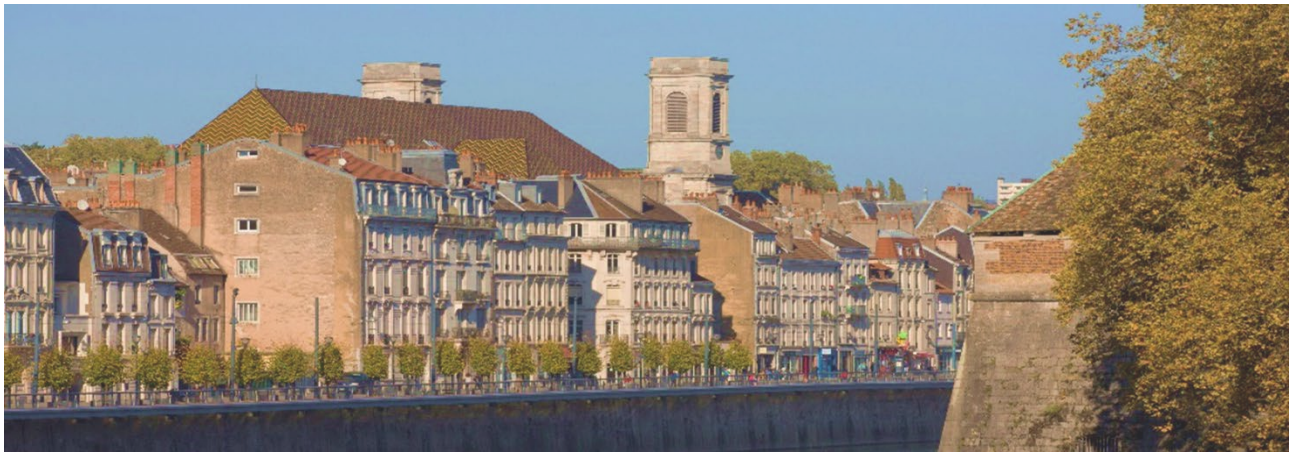
9TH INTERNATIONAL SYMPOSIUM ON ANTIMICROBIAL PEPTIDES

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OPENING LECTURE

Tiny heroes for immense challenges: the next global challenge

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SESSION I

ANTIMICROBIAL PEPTIDE RESISTANCE

KEYNOTE

Host defence peptide-induced antimicrobial tolerance in *Staphylococcus aureus*

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Resistance and tolerance to antimicrobial peptides in Gram-negative bacteria: roles for cell envelope and stress response modulation

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Introduction

Antibiotic resistance is an increasing concern for modern medicine, making treatment of infections caused by resistant bacteria very tedious. But it is not the only threat: bacteria not intrinsically resistant to an antibiotic can still survive antibiotic treatment, possibly resulting in chronic infections. These bacteria are referred to as tolerant bacteria and are thought to exhibit low metabolic activity, resulting in slow growth. Classical antibiotics are inefficient towards these bacteria, since they usually target pathways related to bacterial proliferation. In contrast, antimicrobial peptides (AMPs) are membrane-active compounds that have been hypothesized to efficiently target slow-growing bacteria. However, recent evidence indicates that some bacteria can be resistant and/or tolerant to AMPs.

Objectives

We aim at understanding the mechanisms involved in bacterial survival to AMPs, either through resistance emergence or through tolerance. We want to identify the mechanisms involved in these two phenomena to understand whether similarities exist.

Methods

In this project, we first investigated the extent of bacterial resistance emergence, using in vitro resistance selection, towards a collection of 11 AMPs from diverse origins and with diverse modes of action and structures, on both Escherichia coli and Acinetobacter baumannii. Resistance mechanisms were identified by whole genome sequencing and dissected by recreating individual mutations in wild-type strains and testing their effect on AMPs resistance levels as well as potential consequences of these mutations on bacterial fitness and virulence.

In parallel, we assessed the level of tolerance to our collection of AMPs of stationary phase bacteria and amino-acid starved bacteria. A transposon mutant collection of E. coli was built and challenged with AMPs in stationary phase conditions to identify genes involved in bacterial tolerance to AMPs.

Results

We could isolate mutants towards a majority of the tested AMPs. Detected mutations were mostly affecting genes involved in cell envelope biogenesis and modification, and in stress response. We could observe in some cases, that resistance selection to one AMP resulted in cross-resistance to other AMPs, with various consequences on fitness and virulence.

In parallel, we observed that stationary phase bacteria were tolerant to all tested AMPs, but at different levels. In contrast, activating the stringent response to mimic amino acid starvation had variable effect on tolerance level depending on the AMP and the bacterial species that were used, even potentiating the efficacy of some AMPs. We expect that transposon mutagenesis and sequencing (TnSeq) will allow us to identify genes important for tolerance to AMPs.

Conclusions

Gram-negative bacteria can not only develop resistance to AMPs, but can also adapt to survive their presence, becoming tolerant to them. Results obtained here indicate that envelope biogenesis and stress response may be important players in bacterial adaptation to AMPs. However, different growth inhibitory signals (stationary phase vs amino acid starvation) may result in different outcomes regarding bacterial adaptation to AMPs. These results are promising and we hope to identify pathways which modulation could lower both resistance emergence and tolerance to AMPs, potentially improving the efficacy of these peptides.

Limited and delayed adaptation to a proline-rich antimicrobial peptide in *Francisella novicida*

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Introduction:

Francisella tularensis is a highly virulent intracellular Gram-negative bacterium and the causative agent of tularemia. Due to its high infectivity, potential for aerosol transmission and high virulence, *F. tularensis* represents a major biological threat[1]. Although *F. tularensis* remains susceptible to currently recommended antibiotics, understanding its capacity to adapt under antimicrobial selective pressure is essential to anticipate the emergence of resistance and support the development of novel therapeutic strategies[2]. Antimicrobial peptides (AMPs) are promising alternatives to conventional antibiotics, notably because of their diverse mechanisms of action and potentially lower propensity for resistance development[3]. In this context, we investigated proline-rich antimicrobial peptides (PrAMPs), a distinct class of AMPs acting primarily on intracellular bacterial targets[4]. We therefore sought to determine the evolutionary robusteness of Pr1, a PrAMP active against *Francisella* spp, under prolonged selective pressure.

Objectives:

This study aimed to characterize the emergence and dynamics of adaptation to Pr1 in *F. novicida*, used as a BSL-2 surrogate, and to compare them with adaptation observed under doxycycline selective pressure.

Further investigations aim to identify the genetic determinants and potential fitness costs associated with Pr1 adaptation.

Methods:

Independent *F. novicida* populations were serially passaged under increasing selective pressure with Pr1 or doxycycline. Changes in susceptibility were monitored throughout experimental evolution by MIC determination. Pr1-adapted populations are being further characterized through clonal isolation and whole-genome sequencing to identify genetic alterations associated with reduced susceptibility. The fitness of adapted clones will also be investigated to determine whether adaptation to Pr1 is associated with an evolutionary cost.

Results:

Pr1 exhibited antibacterial activity against *F. novicida*. During serial passage experiments, adaptation to Pr1 was heterogeneous among independently evolved populations. Only one of four populations developed a marked increase in Pr1 MIC, which occurred after prolonged exposure. In contrast, resistance to doxycycline emerged earlier and more consistently under the experimental conditions tested. Preliminary whole-genome sequencing of the Pr1-adapted population identified a deletion disrupting a gene belonging to the ABC transporter ATP-binding family, resulting in a predicted loss of function. This alteration may contribute to reduced Pr1 susceptibility by limiting peptide uptake, suggesting that intracellular access may represent an important determinant of Pr1 activity. Further clonal characterization is underway to confirm the association between this mutation and the adapted phenotype and to determine whether Pr1 adaptation is associated with a fitness cost.

Conclusions:

These findings reveal distinct adaptive trajectories under Pr1 and doxycycline selective pressures. Under the conditions tested, reduced susceptibility to Pr1 emerged less consistently and later than resistance to doxycycline, suggesting a more constrained evolutionary response to Pr1.
The identification of a mutation affecting a membrane transporter further points to peptide uptake as a potential determinant of Pr1 activity and resistance. Ongoing genomic and phenotypic characterization of adapted clones will provide further insight into the mechanisms and evolutionary consequences of Pr1 adaptation.

Together these results support the interest of Pr1 as afn antimicrobial candidate while highlighting peptide uptake as a key factor to consider in resistance development.

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SESSION II

DISCOVERY AND BIOSYNTHESIS OF ANTIMICROBIAL PEPTIDES

KEYNOTE

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Biosynthesis and Functional Characterization of Novel Type II Sactipeptides from the Human Gut Microbiome

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Introduction

Antimicrobial resistance is a major global health threat, projected to cause over 10 million deaths annually by 2050, highlighting the urgent need for new antibacterial molecules with novel structures and mechanisms of action. Ribosomally synthesized and post-translationally modified peptides (RiPPs) are promising candidates owing to their remarkable chemical and structural diversity. Among them, sactipeptides are a distinctive subgroup, characterized by sulfur-to-α-carbon thioether crosslinks, of which only a limited number have been functionally characterized. We recently characterized Ruminococcin C (RumC1-5), a subclass of sactipeptides produced by *Ruminococcus gnavus* E1, a human gut symbiont. RumC1, the most promising isoform, adopts a unique 3D-structure stabilized by four intramolecular thioether linkages introduced by a radical-SAM enzyme. It exhibits potent antibacterial activity against Gram-positive pathogens in vitro and effectively protects mice from lethal *C. perfringens* infection in vivo, outperforming conventional antibiotics. Importantly, RumC1 displays low cytotoxicity and limited propensity to induce bacterial resistance, highlighting its therapeutic potential and motivating efforts to expand the currently limited repertoire of type III sactipeptides.

Objectives

This study aimed to expand the repertoire of type III sactipeptides by identifying and characterizing RumC-like sequences recovered from the human gut metagenome.

Methods

A large-scale (meta)genomic and bioinformatic screening of the human gut microbiome was performed using RumC sequences as benchmarks. Novel sequences were selected based on stringent criteria, including the number and positions of cysteine residues and the presence of a conserved radical SAM maturase. Selected peptides were produced and purified, and their mature forms were characterized by LC-MS/MS. Their multifunctional properties, including antibacterial activity, cytotoxicity, and anti-inflammatory properties, were subsequently evaluated.

Results

Three of the selected peptides displayed strong antibacterial activity against both Gram-positive and Gram-negative pathogens, although with a narrower spectrum than the model peptide RumC1. None showed detectable cytotoxicity toward intestinal (Caco-2) or hepatic (HepG2) cells or toward erythrocytes. The three peptides also exhibited slight but interesting anti-inflammatory properties.

Conclusions

Overall, these findings identify RumC-like peptides as an underexplored and structurally diverse group of sactipeptides within the human gut microbiome, supporting the potential of radical SAM-dependent RiPPs as promising candidates for the development of next-generation of anti-infectious therapies.

Keywords

Antimicrobial resistance, gut microbiome, antimicrobial peptides, RiPPs, Sactipeptides

Optimization and Functional Characterization of CLEC3A-Derived Antimicrobial Peptides

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Introduction

The increasing prevalence of antimicrobial resistance and difficult-to-treat infections highlights the need for alternative antimicrobial strategies. Antimicrobial peptides (AMPs) are promising therapeutic candidates due to their broad-spectrum activity and potential to combat drug-resistant pathogens. We previously identified the CLEC3A-derived peptides HT-16, HT-47, and WRK-30 as potent anti-infective candidates.

Objectives

This study aimed to systematically optimize CLEC3A-derived antimicrobial peptides through rational sequence modifications and to determine how these modifications affect antimicrobial activity and biocompatibility. In addition, the in vivo efficacy of the antimicrobial peptides HT-47 and WRK-30 was evaluated in murine *Staphylococcus aureus* wound infection models.

Methods

CLEC3A-derived peptides were modified by lysine-to-arginine substitutions, C-terminal methylation, and sequence truncation. Antimicrobial activity against *Staphylococcus aureus* and *Candida auris* was assessed using viable-count and metabolic-activity assays. Peptide-induced ultrastructural changes in fungal cells were investigated by scanning and transmission electron microscopy. Biocompatibility was assessed by cytotoxicity assays in NIH/3T3 murine embryonic fibroblasts and PMA-differentiated THP-1 macrophage-like cells and by hemolysis assays using murine and human erythrocytes. In vivo efficacy was investigated in murine *S. aureus* wound infection models using contaminated sutures placed in open or subcutaneous wounds. Peptides were continuously delivered via osmotic pumps, followed by analysis of bacterial burden in wound tissue, differential blood counts, and systemic cytokine profiles. Explanted sutures were additionally examined by scanning electron microscopy to assess peptide-induced morphological changes in adherent bacterial cells.

Results

Modified peptides derived from HT-16 showed enhanced antimicrobial activity against both *S. aureus* and *C. auris*, whereas modifications of the other CLEC3A-derived peptides generally retained potent antimicrobial activity against both pathogens. Most peptides showed a favorable biocompatibility profile, with no significant hemolytic activity and only selected variants reducing cell viability at higher concentrations. In the murine *S. aureus* wound infection models, viable-count analysis showed a trend toward reduced bacterial burden in wound tissue following treatment with HT-47 and WRK-30. SEM analysis of explanted contaminated sutures revealed morphological alterations of adherent bacterial cells consistent with peptide-induced bacterial damage. No treatment-related alterations were observed in differential blood counts or systemic cytokine profiles.

Conclusions

Our findings demonstrate that rational modification of CLEC3A-derived antimicrobial peptides can enhance antimicrobial activity while maintaining a favorable biocompatibility profile. The observed differences in antimicrobial activity, cytotoxicity, and hemolysis highlight the importance of evaluating peptide optimization beyond antimicrobial potency alone, while the trend toward reduced bacterial burden and the observed morphological alterations of adherent bacteria *in vivo* support further evaluation of selected CLEC3A-derived peptides as anti-infective candidates.

A cell-free DNA-to-bioactive platform for rapid discovery of natural peptide lantibiotics enables gut eradication of vancomycin-resistant *Enterococci*

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Introduction

The expansion of microbial genome and metagenome sequencing has shifted antimicrobial peptides (AMPs) discovery from molecule-first to sequence-first. Genome mining now predicts numerous AMP biosynthetic gene clusters (BGCs), but experimental validation remains a major bottleneck. Most candidates still require native fermentation, heterologous expression or chemical synthesis, which are slow, labor-intensive, and low throughput.

Objectives

This bottleneck is particularly severe for ribosomally synthesized and post-translationally modified antibacterial peptides (RiPPs), including nisin-like lantibiotics [1], because their small, highly modified cyclic structures are far more difficult to synthesize chemically than linear AMPs. Nisin-like lantibiotics are a clinically important RiPP family, yet most predicted variants remain functionally uncharacterized. Beyond antibacterial potency, translational development also requires pathogen selectivity to minimize microbiome disruption and improved resistance to enzymatic degradation. Their diversity arises from variable precursor peptides and tailoring enzymes, but functional characterization is often hindered by host incompatibility, regulatory complexity, precursor toxicity, instability, and incomplete maturation in vivo. As a result, many predicted BGCs remain silent, and few candidates are tested at scale against high-priority pathogens such as vancomycin-resistant *enterococci* (VRE). We aimed to build a generalizable sequence-to-function platform for the rapid production and screening of mature AMPs, with a specific focus on nisin-like lantibiotics.

Methods

We developed a modular cell-free protein synthesis (CFPS) workflow integrating: (i) bioinformatic AMP precursor prioritization, (ii) in vitro precursor expression by CFPS, (iii) enzyme-guided post-translational maturation, and (iv) direct antibacterial testing. This design enables rapid, parallel conversion of predicted BGC outputs into testable molecules, reducing the design–build–test cycle from weeks to day.

Results

Using the minimal nisin biosynthesis framework [1], we combined antiSMASH with our in-house SPECO [2] pipeline to prioritize precursors containing the motif xxS/Txn$_{(3–4)}$CS/TxxC (X = any amino acid) plus the key modification enzyme NisB. Analysis of >280,000 genome and metagenome datasets, followed by core-peptide deduplication, yielded >300 unique nisin-like lantibiotic core peptides. CFPS screening identified antibacterial activity in >150 candidates. Heterologous expression and mass spectrometry of bioactive hits confirmed substantial structural diversity and a broad range of antibacterial spectra.

Two lead compounds were purified at scale: a five-ring peptide and a four-ring peptide. Both exhibited potent, narrowerspectrum activity against *Enterococcus faecalis*, including VRE, compared with nisin A and daptomycin, with no detectable hemolysis or toxicity. Additionally, the five-ring peptide showed intrinsic resistance to gut proteolytic enzymes, with all potential cleavage sites naturally mutated. Consistent with this enhanced gastrointestinal stability, oral gavage in a mouse intestinal colonization model reduced VRE burden in vivo, supporting its suitability for gut-targeted antienterococcal therapy. Mechanistic assays indicated distinct actions; notably, the four-ring peptide did not form membrane pores.

Conclusions

We developed a scalable cell-free protein synthesis pipeline that connects genome mining to rapid functional discovery of nisin-like lantibiotics. From >280,000 (meta)genomes, we identified >300 unique cores and found antibacterial activity in >150 candidates. Two lead peptides showed strong and narrower-spectrum activity against *E. faecalis* (including VRE), no detectable hemolysis/toxicity, and distinct mechanisms. Oral delivery also reduced gut VRE colonization, highlighting translational potential for peptide antibiotic discovery.

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Heterologous expression as a powerful tool for the production and discovery of antipseudomonal and antimycobacterial peptide natural products

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Introduction

Bacterial natural products play an important role in the development of pharmaceutical drugs. *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* are two human pathogens; *P. aeruginosa* causes severe nosocomial infections such as ventilator-associated pneumonia and urinary tract infections, while *M. tuberculosis* causes tuberculosis—one of the world's deadliest infectious diseases, responsible for approximately 1.2 million deaths every year. Uridyl peptide antibiotics (UPAs) exhibit potent activity against *P. aeruginosa* by inhibiting the underexplored target phospho-*N*-acetylmuramyl-pentapeptide translocase (MraY), a protein that catalyses the first membrane-bound step in bacterial cell wall biosynthesis.[1] Lasso peptides are a family of extraordinarily stable ribosomally synthesised and post-translationally modified peptides (RiPPs). Two representatives, lassomycin and kitamycobactin, have been identified as promising antimycobacterial agents, targeting the ClpC1P1P2 protease complex of mycobacteria.[2,3]

Objectives

The primary focus of our research group is the identification and heterologous production of novel natural products that exhibit interesting bioactivities. UPAs are produced by different streptomycetes, but the production in wild-type strains and several heterologous hosts is low and has halted the further development of this class. Therefore, an efficient heterologous production platform for one of the most potent UPAs, mureidomycin A, needs to be created. In addition to producing antipseudomonal natural products, we apply the heterologous approach to discover antimycobacterial natural products, i.e. novel lasso peptides with bioactivity against *M. tuberculosis*.

Methods

An interdisciplinary approach is employed, encompassing the utilisation of bioinformatic tools (e.g. genome mining, antiSMASH, AlphaFold), molecular biological and biochemical methods (e.g. cloning, RT-PCR, heterologous expression of proteins and full gene clusters, enzyme characterisation), and chemistry-based approaches (e.g. activity-guided isolation of natural products, structure elucidation).

Results

Two UPA clusters were engineered to establish a heterologous production platform for mureidomycin A. For this purpose, the gene clusters were modified by non-ribosomal peptide synthetase (NRPS) engineering to mainly produce mureidomycin A. Furthermore, gene deletions and promoter tuning was performed to eliminate the production of less active compounds and enhance mureidomycin A production. These modifications increased the yield of mureidomycin A by approximately two orders of magnitude, with the final strain producing ~1.4 g/L.[1,4]

Bioinformatic analyses have identified *Kitasatospora* sp., *Streptomyces* sp., and *Nocardioides* sp. as lasso peptide producers. A novel producer strain was identified for the known compound kitamycobactin. Since the lasso peptide gene clusters from *Streptomyces* sp. and *Nocardioides* sp. are silent, the corresponding biosynthetic gene clusters were expressed heterologously, which allowed the identification of two novel lasso peptides. Preliminary bioactivity tests confirm the expected antimycobacterial activity. Furthermore, the methyltransferases situated in these gene clusters were characterised.

Conclusions

Bacterial natural products are highly versatile compounds with a variety of applications, including drug development for patient treatment. Heterologous expression is a powerful tool in this process, enabling the production of a broad range of natural products. This is particularly useful when biosynthetic gene clusters are silent in the native producer, as it often allows the identification of the encoded natural products.

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SESSION III

INNOVATION, ARTIFICIAL INTELLIGENCE, MACHINE LEARNING IN ANTIMICROBIAL PEPTIDES

KEYNOTE

AI-Driven Discovery of Cryptic Antimicrobials from the Human Microbiota

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The rise of antibiotic-resistant pathogens poses an urgent global health threat, yet traditional discovery platforms have largely stagnated. The human microbiota—an untapped reservoir of bioactive natural products—offers a promising solution. These microbial communities produce diverse antimicrobial compounds that mediate inter-microbial competition and host–microbe communication. To harness this potential, we present an interdisciplinary platform that integrates artificial intelligence and synthetic biology to decode the cryptic chemistry of the microbiota for antimicrobial discovery. AI-driven deep learning models first mine massive meta-omics datasets to decode the biosynthetic potential of antimicrobial peptides (AMPs). These computational predictions are then experimentally validated and scaled through synthetic biology, chemical synthesis, and high-throughput screening. Beyond antimicrobial discovery, we apply AI to decode the chemical ecology of host–microbe interactions, mapping the molecular "language" that governs microbial behavior and resilience. By linking ecological insights to therapeutic design, we identify not only new antimicrobials but also engineer probiotic systems capable of disrupting pathogenic networks or modulating host immunity. This AI-to-biology strategy bridges fundamental ecology with translational innovation, offering a scalable roadmap to combat resistance and pioneer next-generation antimicrobial therapies.

AI-Guided Discovery and Probiotic Delivery of *Enterococcus*-Targeting Bacteriocins for Pathogen Control and Intestinal Barrier Repair

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Introduction

Antibiotic exposure can promote intestinal domination by vancomycin-resistant enterococci (VRE), while compromised epithelial barriers facilitate bacterial translocation and invasive infection. Broad-spectrum antimicrobial treatment may further disrupt microbiota homeostasis, highlighting the need for more selective interventions. Engineered probiotics offer a route for local antimicrobial production and complementary mucosal repair, particularly during the actionable window between enterococcal overgrowth and progression to invasive disease in antibiotic-exposed patients.

Objectives

We aim to develop an inflammation-responsive "sense-and-kill" probiotic that combines targeted enterococcal suppression with intestinal barrier repair. Class IIa bacteriocins were selected for their relatively narrow antimicrobial spectra and receptor-dependent recognition of the mannose phosphotransferase system (Man-PTS) in susceptible bacteria. Our objectives are to prioritize bacteriocins against target-strain Man-PTS using AI-guided modeling, establish delivery in Escherichia coli Nissle 1917 (EcN), and integrate inflammatory sensing with complementary trefoil factor 3 (TFF3) delivery. Local probiotic production is intended to address peptide instability and delivery limitations, while responsive regulation aims to align therapeutic output with disease-associated conditions.

Methods

A task-specific class IIa bacteriocin–Man-PTS dataset was constructed by integrating genome mining with synthetic biology–informed design principles. Because general-purpose protein AI models may not generalize reliably to strain-specific bacteriocin–receptor recognition, a dedicated predictive model was developed for this interaction context. Sequence embeddings from the pretrained protein language model ESM2 were used to train a neural network, adapting general protein sequence representations to bacteriocin–Man-PTS interaction prediction and target-strain-directed candidate prioritization. The workflow was applied to vancomycin-resistant *Enterococcus faecalis* V583, yielding 12 candidates for experimental evaluation. An EcN bacteriocin expression platform was established using a native-plasmid-based architecture. A calprotectin-associated sensing module was designed to exploit inflammation-linked metal limitation as a regulatory input for bacteriocin production. Complementary TFF3 delivery was included in the platform design to address epithelial injury alongside bacterial burden reduction.

Results

Eight of the 12 AI-prioritized bacteriocin candidates exhibited antimicrobial activity against V583, corresponding to an experimental hit rate of 66.7%. These findings support the utility of the task-specific workflow for identifying experimentally active candidates against the selected enterococcal target. Bacteriocin was detected in engineered EcN culture supernatants, providing preliminary evidence of heterologous production and extracellular availability. The modular expression platform provides a foundation for subsequent integration of inflammation-responsive regulation and barrier-repair functions. A sense-and-kill regulatory architecture was designed, whereas signal-dependent antimicrobial activity, microbiota selectivity, and TFF3-mediated epithelial protection remain to be validated.

Conclusions

AI-guided, receptor-informed prioritization identified multiple bacteriocins active against VRE, and production in EcN supports the feasibility of probiotic-based delivery. By combining selective antimicrobial candidates with inflammation-associated regulation and mucosal repair, this platform seeks to address both enterococcal overgrowth and residual barrier injury. Subsequent studies will determine whether the integrated approach improves bacterial burden and epithelial integrity outcomes compared with either intervention alone, while assessing its potential to limit disruption of the surrounding intestinal microbiota.

Uncovering Antimicrobial and Immunomodulatory Effects of Natural and Encrypted Peptides from the Mexican Axolotl using Machine Learning and AI

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Among the new therapeutic strategies developed as alternatives to combat antibiotic-resistant infections are host defence peptides (HDPs). These components of the innate immune system in eukaryotes are characterized by their short length (12 to 50 amino acids), cationic and amphipathic nature, and high content of hydrophobic residues (approximately 50%), with most adopting an alpha-helical structure. Because most of the HDPs induce microbial death by interacting with membranes through electrostatic and hydrophobic interactions, leading to membrane disruption, they have been shown to generate less bacterial resistance than conventional antibiotics. In addition to their antimicrobial activity, they often exhibit other biological functions, such as antitumor effects and modulation of the immune response.

These include promoting chemotaxis, phagocytosis, apoptosis, cell proliferation, and the production of cytokines, chemokines, and reactive oxygen species. This immunomodulatory effect combined to direct antimicrobial effect could be promising in the treatment of infections. HDPs can be produced through both classical ribosomal synthesis and proteolytic processing. The latter has gained increasing interest in recent years due to the discovery of novel antimicrobial peptides, known as cryptic host defence peptides. With the aid of computational methods, algorithms can be applied to scan the proteome for these cryptic peptides based on HDPs characteristics such as sequence length, net charge, average hydrophobicity, and other physicochemical properties, all integrated into a fitness function. Moreover, machine learning and Artificial Intelligence can help characterize desired biological activities.

Although amphibians constitute the largest known group of organisms with identified and characterized HDPs exhibiting antimicrobial and immunomodulatory activity, very little is currently known about the host defence peptides of the Mexican axolotl (*Ambystoma mexicanum*). In order to discover new therapeutic candidates, we used the “classical” technique of skin exudate purification and the computational methods on the whole proteome to identify some new peptides and to predict their biological activities (i.e immunomodulatory, antimicrobial and potential cytotoxic effects. The biological activities predicted were verified by in vitro experiments such as antimicrobial, immunomodulatory and cytotoxic assay.

In conclusion, we identified various new peptides either with the classical method or with the computational method.

Discovering novel antimicrobial peptides in the Animal Kingdom

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The evolutionary origins of antimicrobial peptides (AMPs) remain poorly studied. These highly diverse, fast evolving short amino acid sequences are difficult to uncover using homology approaches, as many of them appear to be taxonomically restricted. In eukaryotes, most AMPs are only a part of the larger precursor protein, which is cleaved during “maturation”. As machine learning classifiers have been trained on mature peptides, their precise coordinate prediction in the larger sequence is of essence when trying to identify them in silico. Their tertiary structures are simple due to their small size, yet the full extent of the different possible conformations and their structural evolution is largely overlooked.

In this study we are trying to explain the extreme diversity that characterizes AMPs. Could they originate de novo from previously noncoding sequences, or is it through divergence? Creating a large catalogue of curated candidate AMPs (c-AMPs) can solve their sparse representation in the amino acid sequence space, thus allowing us to understand their modes of origin. Can their structures provide enough information for these short sequences, and does this diverse group of peptides provide a good medium for novel fold emergence? Our study aims to answer all the above.

We present a computational mining pipeline capable of predicting high-confidence novel c-AMPs from MATEdb2 database’s metazoan genomes and transcriptomes, using 3 peptide coordinate prediction tools alongside 5 AMP classifiers. Clustering followed by extensive sequence similarity searches of the resulting c-AMPs were used to identify those being novel, taxonomically restricted, including those unique to a single species. We are currently working on inferring the processes by which novel AMPs originate through comparative genomic analysis, with particular focus on de novo gene emergence cases. State-of-the-art protein language models predicted tertiary structures for these candidates, and structural segmentation and similarity tools were used to obtain domain and fold level annotations. Lastly, to validate our predictions and the performance of the pipeline, 82 novel candidates were synthesized and tested in vivo against 4 relevant bacterial species.

More than 32,000 high confidence c-AMPs were predicted, with total agreement between all classifiers, of these approximately 18,000 are novel. Structural analysis of these novel candidates revealed similarities with other AMPs or structures in public databases. Additionally, this resulted in high quality novel folds and domains, providing a valuable dataset for investigating novel fold emergence and potentially new protein-protein interactions. 67% of the synthesized c-AMPs exhibit positive antimicrobial function, some of which display broad-spectrum activity. As we tested mostly novel species-specific candidates, this result speaks volumes on the pipeline’s ability to predict AMPs.

In conclusion, our study more than doubles the AMP family sequence space, providing insights regarding their evolutionary origins. Combined with the structural annotations of these peptides, the resulting catalogue can be advantageous for future AMP research.

SESSION IV

MICROBIAL ECOLOGY, MICROBES AND INTERACTIONS WITH THEIR HOSTS

KEYNOTE

Beyond Antimicrobial Activity: PAX Cyclolipopeptides Have Multifaceted Roles in Xenorhabdus–Nematode–Insect Interactions

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Two antimicrobial peptides in host-symbiont interactions: functional analysis in the rice weevil *Sitophilus oryzae*, a major insect pest of stored cereals, and the bacterium *Sodalis pierantonius*

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Many insects that feed on nutritionally unbalanced environments maintain a mutualistic symbiotic relationship with intracellular bacteria (endosymbionts) that supplement their nutritional intake, e.g. in vitamins and essential amino acids, which hosts cannot find in their unbalanced diet nor synthesize themselves. In these insects, endosymbionts are compartmentalized in specialized host cells called bacteriocytes, which often group into organs, the bacteriomes. In the bacteriocytes, bacteria remain metabolically active and provide essential nutriments to the host. The vertical transmission of these intracellular bacteria over generations of insects fosters a long-term coevolution with the host, which is often associated with genome degenerescence. In contrast, the Gram-negative bacteria Sodalis pierantonius has been relatively recently associated with the cereal weevil (*Sitophilus spp*), after the replacement of a more ancestrally-associated endosymbiont. *S. pierantonius* has conserved many traits of a free-living bacteria, including the capacity to produce a cell wall.

In two cereal weevil species, *Sitophilus oryzae* and *Sitophilus zeamais*, antimicrobial peptides (AMPs) are involved in the elimination of Gram-negative bacteria after a systemic infection, through the activation of the IMD immune pathway, which is complete and functional (Maire et al. 2018 ; Parisot et al. 2021). In addition to this function in the immune response to free-living bacteria, some AMPs are also involved in the compartmentalization (i.e. the restricted location) of the endosymbionts in the bacteriocytes. Previous functional studies in S. zeamais demonstrated that in the absence of the AMP Coleoptericin A (ColA) endosymbionts escape out of the bacteriocytes, demonstrating that ColA is essential for maintaining symbiont under the host's control, without killing them (Login *et al*. 2011).

More recently, extensive transcriptomic data performed in *S. oryzae* showed that not only ColA but also another AMP, Diptericin-like (Dpt-like) is highly expressed in bacteriomes (Ferrarini *et al*. 2023), strongly suggesting a function in the regulation of symbiosis in S. oryzae. Here we will present the functional analysis of both colA and dpt-like in *S. oryzae*, through the study of their expression under different conditions and the characterization of the phenotypes generated by the inhibition of their expression via RNA interference (RNAi).

Antimicrobial peptides as signalling molecules in gut bacteria

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In the gut, effectors of the innate immune system such as antimicrobial peptides (AMPs) affect bacterial survival in a concentration-dependent manner. Given their therapeutic potential, most studies have focused on their bactericidal activity at lethal concentrations. *In vivo*, however, secreted AMPs do not necessarily reach inhibitory levels, as local concentration gradients naturally occur. The few studies performed at sub-inhibitory concentrations, mainly on pathogens,report modulation of bacterial signalling, including biofilm formation and virulence. The underlying cellular and molecular mechanisms remain largely unknown, despite their physiological relevance.

This project aims to determine whether AMPs function as signalling molecules and to characterise the responses they elicit in gut bacteria at physiologically relevant concentrations.

Quantitative proteomics of *E. coli* K-12 MG1655 exposed to sub-inhibitory concentrations of cationic AMPs of distinct origins (human LL-37 and the synthetic peptide C18G) revealed a substantial proteomic response. The response was strongly peptide-specific, and functional enrichment analysis identified modulation of pathways involved in central metabolism, biofilm formation, motility, chemotaxis and AMP resistance. Notably, approximately 60% of the regulated proteins are functionally uncharacterised, underscoring the need for mechanistic investigation.

Phenotypic assays confirmed biofilm formation and quorum sensing were differentially modulated by the two peptides. Screening of deletion mutants of selected candidate genes identified eight strains with altered growth in the presence of AMPs. Among them, a mutant lacking a conserved protein of unknown function was completely growth-inhibited by both LL-37 and C18G at otherwise sub-inhibitory concentrations. This mutant also displayed abnormally short cells, suggesting an impairment of cell division or separation. Together, these results support a signalling function for AMPs at sub-inhibitory concentrations, advance the understanding of bacterial response mechanisms, and identify a conserved protein of unknown function required for tolerance to LL-37 and C18G.

Candidalysin targets bacteria: a possible role in fungal-bacterial competition

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Candida albicans is an opportunistic pathogen causing superficial epithelial and invasive infections. Its virulence is tightly linked to the secretion of candidalysin (CL), a 31-amino-acid amphipathic, α-helical peptide toxin that damages host cells[1], notably epithelial cells, by inducing membrane permeabilization through pore formation[2]. In the host, *C. albicans* predominantly co-exists with diverse bacterial species in complex communities across mucosal niches. Depending on the bacterial species, these interactions can be synergistic or antagonistic and play an important role in polymicrobial infections[3]. While the effects of CL on host cells are well characterized, its impact on the surrounding bacterial microbiota remains largely unexplored.

Given the limited knowledge of the role of CL in *C. albicans*-bacteria interactions, this work aims to provide a multiscale mechanistic analysis of the effects of CL on bacterial cells, comparing Gram-positive and Gram-negative species. We addressed three questions: (i) how do bacteria respond to CL exposure? (ii) Does CL interact directly with bacterial cells, and what are the consequences at the cellular and nanoscale levels, including changes in cell morphology, plasma membrane integrity, surface ultrastructure, and the nanomechanical properties of the cell envelope? and (iii) How does CL perturb model lipid membranes that mimic bacterial membranes at the nanoscale?

To this end, we combined biophysical approaches (e.g., atomic force microscopy) with microbiological assays to probe the effects of CL on *Staphylococcus aureus* and *Escherichia coli*, linking single-cell and nanoscale changes to population-level phenotypes.

CL induced concentration-dependent aggregation in both *S. aureus* and *E. coli*, with species-specific dispersibility: *S. aureus* formed stable aggregates (6 to 30 μm²), whereas *E. coli* aggregates were readily dispersed by mechanical disruption. Immunofluorescence confocal imaging showed that CL binds to the bacterial cell surface and colocalizes with the plasma membrane, with a stronger association for *S. aureus* (Pearson's correlation coefficient, PCC = 0.7) than for *E. coli* (PCC = 0.5), suggesting that direct CL-bacteria interaction contributes, at least in part, to aggregation. This preferential surface accumulation was followed, after prolonged exposure, by marked bacterial envelope alterations. CL (i) compromised plasma membrane integrity in both species;(ii) induced pronounced morphological changes in *S. aureus*, notably an increased cell diameter, whereas *E. coli* showed only slight size variations; (iii) altered the cell surface ultrastructure, increasing the RMS roughness 5-fold in *S. aureus* and 3-fold in *E. coli*; and (iv) reduced cell envelope elasticity 2- to 3-fold, indicating pronounced cell softening. Finally, CL induced nanoscale defects of varying depth, typically 30 to 50 nm in lateral dimension, in bacterial membrane bilayer models: mostly membrane thinning (<1 nm deep) or hemipores (1 to 2 nm deep), and less frequently partial transmembrane defects (2 to 3 nm deep). CL also caused complete membrane removal (~5 nm deep) and exposing the underlying substrate.

Together, these findings show that CL, although primarily described as a fungal cytolytic toxin involved in pathogenicity towards host cells, acts on bacteria in a manner similar to membrane-active antimicrobial peptides[4,5], suggesting that it may contribute to *C. albicans* competition within polymicrobial communities.

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SESSION V

PEPTIDE DESIGN AND BIOENGINEERING

KEYNOTE

Chemical Space Guided Design of Antimicrobial Peptides

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Multidomain engineering of bovine host defense peptides for broad-spectrum antimicrobial applications

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The emergence of antibiotic-resistant pathogens demands new treatment strategies. Bovine host defense peptides (bHDPs) play a major role in the innate immunity of cattle and hold potential applications against a broad range of pathogens. Recombinant production offers a scalable and cost-effective method for their manufacture. However, the short size of HDPs makes them highly susceptible to protease degradation during the process. To overcome this limitation, we propose a novel protein-engineering approach for bHDPs based on the fusion of concatamers of identical or different peptides in a single sequence, generating larger products with improved stability and activity. This approach aims to expand the applicability of bHDPs against relevant antibiotic-resistant pathogens and complex polymicrobial diseases, such as bovine respiratory disease (BRD).

To generate multidomain bHDPs constructs, bovine β-defensin 1 (bBD1), bovine β-defensin 3 (bBD3), lingual antimicrobial peptide (LAP) and bactenecin 5 (Bac5) were combined and recombinantly produced in *Escherichia coli*. The resultant proteins were bBD1, bBD1X2, bBD1X3, bBD1X4, bBD1LAPX2 and bBD31LABA. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were performed against *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus epidermidis* (MRSE) with the proteins alone and combined with different antibiotics. The combinations were also performed against the pathogens in biofilm state. In addition, the proteins were tested against bacterial BRD pathogens by CFU counting method and for viral BRD pathogens by an ATP detection method that measured the survival of infected cells.

All multidomain proteins were successfully produced and showed activity against MRSE and *P. aeruginosa*. Furthermore, they were more active and soluble when the protein contained four HDPs, as demonstrated by comparison of bBD1, bBD1X2, bBD1X3 and bBD1X4. When combined with antibiotics, synergistic effects were only observed between bBD31LABA and protein synthesis inhibitors against P. aeruginosa. This synergy appeared to be associated with a strong outer-membrane permeabilization activity of bBD31LABA, which may facilitate the uptake of antibiotics such as chloramphenicol and gentamicin. Contrary to this pattern, the best combination against *P. aeruginosa* biofilms was bBD31LABA and meropenem, a cell wall synthesis inhibitor, suggesting that interactions observed in planktonic cultures do not necessarily predict those occurring in biofilms. Against BRD pathogens, high antibacterial and antiviral effects were achieved, particularly with bBD31LABA. This protein showed strong activity against *Pasteurella multocida* and *Mannheimia haemolytica*, reducing bacterial cell counts to undetectable levels and by 3 log CFU/mL, respectively, after 4h incubation. The activity was moderate against *Histophilus somni* and *Mycoplasma bovis* but improved when combined with oxytetracycline against *H. somni* and tulathromycin against *M. bovis*. Antiviral activity was also observed against parainfluenza-3 virus, bovine herpesvirus-1 and bovine respiratory syncytial virus (BRSV). Notably, against BRSV infecting bovine turbinate cells, bBD31LABA and bBD1LAPX2 exhibited even growth-promoting properties, reaching 136% and 128% cell viability, respectively, at their highest concentrations tested.

Overall, multidomain engineering of bHDPs resulted in the generation of broad-spectrum proteins capable of targeting relevant human antibiotic-resistant bacteria and multiple bacterial and viral pathogens involved in BRD. Furthermore, their membrane-permeabilizing activity and antibiofilm properties highlight their potential to enhance the efficacy of conventional antibiotics.

Omp38 inclusion bodies for engineering long-lasting antibiofilm surfaces

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Introduction

Staphylococcus epidermidis is a major cause of device-associated infections, largely due to its ability to colonise abiotic surfaces and establish persistent biofilms, which reduce susceptibility to antimicrobial treatment. Preventing bacterial colonisation before a mature biofilm is established therefore represents an attractive complementary strategy to conventional antimicrobial approaches. The development of such preventive strategies depends not only on identifying bioactive proteins, but also on converting them into stable functional materials. Inclusion bodies (IBs), protein nanoparticles generated during recombinant production, can retain biologically active protein species and progressively release functional protein, making them promising building blocks for antimicrobial and antibiofilm surface engineering. Here, we explored whether the intrinsic aggregation propensity of Omp38, an antibiofilm outer membrane protein from the Antarctic bacterium *Psychrobacter* sp. TAE2020, could be directly exploited to generate a stable antibiofilm material.

Objectives

We aimed to evaluate the potential of Omp38 IBs as functional components of antibiofilm surfaces to prevent or eradicate biofilm formation produced by two different *Staphylococcus epidermidis* strains.

Methods

Omp38 and the fluorescent Omp38-mScarlet variant were heterologously produced in *Escherichia coli*, where both accumulated mainly in the insoluble fraction, forming IBs. Following IB isolation, proteins were either recovered under non-denaturing conditions and chromatographically purified (soluble protein) or maintained as purified IB preparations. Structural organisation was investigated through spectroscopic and computational approaches. Antibiofilm functionality was evaluated in functionalized surfaces with Omp38 and Omp38-mScarlet (soluble and IBs) against *Staphylococcus epidermidis* reference strain RP62A and the clinical *S. epidermidis* 375 isolate by assessing bacterial adhesion, biofilm development and mature biofilms. VP1GFP IBs were included as a non-functional IB control. Long-term surface functionality was assessed by comparing soluble and IB proteins after prolonged residence on polystyrene surfaces.

Results

Omp38 retained substantial antibiofilm activity when formulated either as a soluble protein or directly as functional IBs. Soluble Omp38 and Omp38-mScarlet showed strong activity against biofilm formation, with maximal effects at 10 µg/mL and biomass reductions of up to 95% and 90%, respectively. Their activity against mature biofilms was more moderate, reaching approximately 40% reduction for Omp38 and 20% for Omp38-mScarlet at 25 µg/mL. Importantly, substantial antibiofilm activity was preserved when both proteins were directly used as purified IBs. At 20 µg/mL, Omp38 and Omp38-mScarlet IBs reduced biofilm formation by approximately 60% and 70%, respectively, while both formulations decreased mature biofilm biomass by around 30%. In contrast, VP1GFP IBs did not reproduce this effect, supporting the conclusion that the observed activity was specifically associated with Omp38 rather than with the IB format itself. Activity was observed against both the reference *S. epidermidis* RP62A strain and the clinical isolate 375. Surface functionalisation further demonstrated the remarkable stability of these protein formulations, with soluble proteins remaining active for up to one month. Notably, the IB forms showed greater preservation of antibiofilm activity over time and significantly improved performance compared with their soluble counterparts following prolonged surface residence.

Conclusions

In conclusion, Omp38 IBs and, to a lesser extent, their soluble version represent a promising new generation of antimicrobial materials that combine intrinsic antibiofilm activity, stability, and prolonged surface functionality.

Strategic Residue Substitution in Tolaasin for the Synthesis of New Bioactive Derivatives

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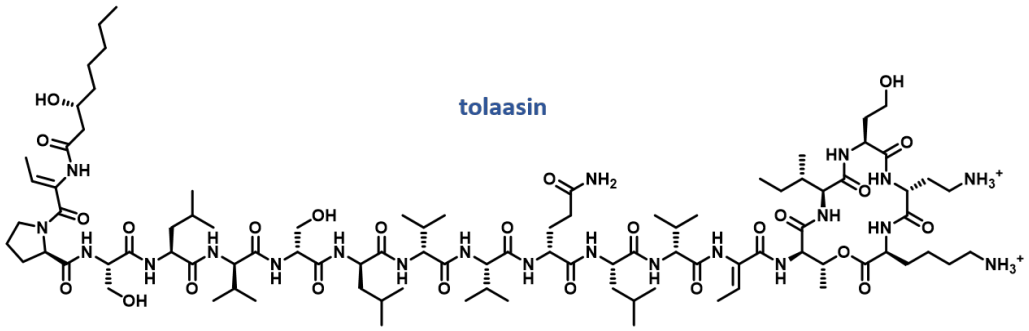
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Tolaasin, an 18 residue cyclic lipodepsipeptide, is the main virulence factor of *Pseudomonas tolaasii*, and causes brown blotch disease in edible mushrooms, indicating antifungal activity [1]. More recently, cytotoxicity against cancer cell lines was also demonstrated. While experimental data supports a mode of action involving direct interaction of tolaasin with cellular membranes, molecular level understanding of the features of tolaasin involved and how these ultimately lead to cell lysis are not well understood [2,3].

Given these intriguing anticancer (and antifungal) properties, a total synthesis route towards tolaasin was successfully developed. After achieving this, we became interested in generating a library of synthetic analogues to further investigate structure-bioactivity relationships for tolaasin.

However, the solid-phase peptide synthesis (SPPS) of tolaasin is hindered by several challenging coupling steps, primarily due to the presence of the non-proteinogenic amino acid *Z*-dehydrobutyrine (*Z*-Dhb) in its sequence. These difficulties significantly reduce the overall yield, resulting in limited amounts of the final peptide.

To overcome this, we introduced a strategic yet straightforward amino acid substitution, replacing these *Z*-Dhb residues. This modification led to the development of a synthetic tolaasin analogue that retains bioactivity comparable to the native compound. Importantly, this substitution substantially reduces synthetic complexity while markedly improving yields, thereby enabling the efficient generation of a library of analogues for comprehensive bioactivity and biophysical studies. Bioactivity screening of a library of derivatives provides a first insight in the molecular determinants essential for bioactivity.



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SESSION VI

MECHANISM OF ACTION OF ANTIMICROBIAL PEPTIDES

KEYNOTE

On the mechanisms of Cell-envelope targeting antibiotics

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Mechanism of action of non-lytic antimicrobial peptides studied by *in vivo* solid-state NMR and fluorescence microscopy

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Antimicrobial peptides (AMPs) can be divided into two classes according to their mechanisms of action. Lytic AMPs kill bacteria by disrupting their membranes. In contrast, non-lytic AMPs kill bacteria by crossing the cell envelope and binding to intracellular targets, thereby inhibiting essential cellular processes, like protein synthesis and folding.

How non-lytic AMPs cross the cell envelope, particularly in Gram-negative bacteria with their two membranes, remains poorly understood. Previous studies have shown that some of these peptides rely on transporters for translocation, whereas others can cross the membrane spontaneously. In this project, we investigate how non-lytic AMPs cross the *Escherichia coli* cell envelope and how they affect the outer and inner membranes.

We use *in vivo* deuterium (^{2}H) solid-state NMR to investigate how the translocation of several non-lytic AMPs, such as buforin and oncocin, affects the lipid order of deuterium-labelled *E. coli* membranes. We also use time-lapse fluorescence microscopy combined with microfluidics to gain insight into the sequence of events triggered by AMPs at the membrane level.

Our results show that most non-lytic AMPs affect membrane permeability, but their effects are less pronounced than those of lytic AMPs. The kinetics of these effects vary considerably between peptides, and their impacts on the outer and inner membranes can also differ. We further show that buforin, a non-lytic AMP capable of spontaneous translocation, significantly affects membrane order.

Metabolomic and proteomic analysis reveals the differential cellular responses of *Bacillus subtilis* to cationic antimicrobial peptides

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Introduction:

Antimicrobial resistance (AMR) has become one of the greatest threats to global health, creating an urgent need for new antimicrobial strategies. Cationic antimicrobial peptides (cAMPs) are promising alternatives to conventional antibiotics because they rapidly kill bacteria through multiple mechanisms, making the development of resistance less likely. However, while their interactions with bacterial membranes have been extensively studied, metabolomic responses remain poorly characterized and appear to strongly depend on the nature of the peptide, the bacterial species, and the exposure conditions.

Objective and methods:

The objective of this work was thus to compare the metabolic and proteomic responses of *Bacillus subtilis* following treatment with two membrane-active AMPs, aurein 1.2 (carpet mechanism) and caerin 1.1 (pore-forming mechanism), and the cell-wall targeting antibiotic vancomycin. *B. subtilis* cells were treated with the antimicrobials at their minimum inhibitory concentrations at the mid-exponential phase of the growth cycle. Intracellular metabolites were then extracted and analyzed by ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry (MS), while protein abundance was determined using LC-HRMS proteomics. Differentially abundant metabolites and proteins were identified using multivariate statistical analysis, and the affected biological pathways were compared across treatments.

Results:

Our results showed distinct, compound-specific cellular responses to the three antimicrobials at both the metabolomic and proteomic levels. Vancomycin induced the most extensive remodeling, characterized by increased nucleotide biosynthesis, central carbon metabolism, and cell envelope associated metabolites, together with decreased amino acid pools. These changes were accompanied by broad proteomic alterations involving stress response pathways, amino acid metabolism, transport systems, and sporulation related processes, consistent with activation of compensatory mechanisms in response to a major cell envelope targeting antibiotic stress. In contrast, aurein produced relatively limited metabolic changes and affected only a restricted set of proteins associated with translation, oxidative stress response, and cytoskeletal organization, supporting a mechanism that primarily disrupts the bacterial membrane without extensive intracellular remodeling. Caerin displayed an intermediate and more complex profile: metabolomics revealed amino acid redistribution but fewer coordinated metabolic changes than vancomycin, whereas proteomics showed broader remodeling involving energy metabolism, redox homeostasis, iron-sulfur cluster assembly, and RNA-associated processes. Together, these results indicate that vancomycin elicits a broad metabolic and proteomic stress response linked to cell envelope disruption, aurein triggers a more constrained response consistent with rapid membrane perturbation, and caerin induces a stronger proteomic adaptation despite moderate metabolic reprogramming.

Conclusion:

To support the development of new peptide-based antibiotics against resistant bacteria, it is important to understand the action mechanism of AMPs and ensuing cellular responses of bacteria. Our innovative integration of metabolomic and proteomic data demonstrate that antimicrobials with different actions towards the bacterial membrane or envelope produce distinct physiological responses despite exhibiting similar antimicrobial efficacy, providing new insight into their mechanisms of action and bacterial adaptation. This work is currently being extended to therapeutically relevant peptides.

Affecting membrane organization to resensitize resistant bacteria to common antibiotics

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Introduction

Antimicrobial resistance is one of the most serious threats to global health. In our approach, we focus on smart combination strategies to resensitize resistant Gram-positive bacteria to common antibiotics like penicillin-binding-protein blocking β-lactams (methicillin, oxacillin) and intracellularly working fluoroquinolones and tetracyclines. Thus, we aim to reuse common antibiotics that are already approved for clinical use worldwide but in time lost their efficacy.

We recently demonstrated that peptidomimetic AMC-109 and lipopeptide N-alkylamide 3d affect the membranes of *Staphylococcus aureus* in an unprecedented way [1, 2]. Antibiotic molecules enter the bacterial membrane and dissolve its organization into functional domains that are essential for bacteria survival. We explore two consequences of this homogenization of the membrane organization. First, oligomers of low affinity penicillin binding proteins (PBP2a) are formed in the membrane domains causing methicillin resistance. Domain dissolution shall thus resensitize bacteria to β-lactams like methicillin and oxacillin. Second, changes in organization and fluidity affect the stable local environment of membrane-bound proteins including efflux pumps. Domains dissolution shall thus decrease the efflux pump activity and resensitize the bacteria to intracellular antibiotics like tetracyclines and fluoroquinolones.

Objectives

To demonstrate that disorganization of the membrane of multidrug resistant *Staphylococci*, in particular dissolution of membrane domains, leads to renewed sensitivity to (i) methicillin and oxacillin, and to (ii) intracellular antibiotics.

Methods

Microbiology checkerboard assays are used to search for the synergistic combinations of membrane-domain-dissolving antibiotics with β-lactams and intracellular antibiotics. Atomic force microscopy provides nano-scale evidence of the antibiotic effects on membrane organization and membrane domain distribution. Fluorescence microscopy and spectroscopy are used to visualize the domain organization in living bacteria and to evaluate the concentration of the intracellular antibiotics inside bacteria.

Results

We successfully found synergistic combinations between a membrane-domain-dissolving antibiotic and both β-lactams and tetracyclines. We show that supplementation with a membrane-domain-dissolving antibiotic indeed lowers the susceptibility of two methicillin-resistant *S. aureus* (MRSA) strains to three different β-lactams several folds. In particular, full reversal of resistance to oxacillin and penicillin in MRSA is achieved. We provide evidence that the β-lactam resistance reversal is indeed linked to dissolution of membrane domains [3]. Similarly, lowered resistance of two *S. epidermidis* strains to tetracyclines was observed when supplemented with a membrane-domain-dissolving antibiotic.

Conclusions

Organization of Gram-positive bacterial membranes was identified as a powerful antibiotic target, both as a standalone target and as a support to resensitize bacteria to other antibiotics. We demonstrate this on two examples — resensitization of MRSA to β-lactams, and of multidrug-resistant S. epidermidis to doxycycline.

[1] Melcrová et al. *Nature Communications* 2023, 14(1), 4038.

[2] Melcrová, Klein, and Roos *Nano Letters* 2024, 24(38), 11800.

[3] Melcrová et al. *BioRxiv*, 2025.

SESSION VII

VECTORIZATION/CLINICAL ENVIRONMENTAL APPLICATIONS

KEYNOTE

Development of the Atrial Natriuretic Peptide as new powerful drug against Pseudomonas aeruginosa biofilms: its mechanism of action and therapeutic potential

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University of Bern, Switzerland

Modulation of bacteriocin-secreting activity through synthetic peptide signaling in *Streptococcus salivarius*

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Introduction

The ability of some commensal bacteria to produce antimicrobial peptides holds promise for future therapeutic applications. Precisely modulating their antimicrobial activity could broaden their potential applications but remains a major challenge. In particular, *Streptococcus salivarius* produces bacteriocins whose expression is regulated through a quorum-sensing system activated by the signaling peptide XIP. This XIP-dependent pathway could therefore be exploited to externally control antimicrobial activity through the addition of synthetic XIP.

Objectives

We investigated how the metabolic environment, XIP concentration and timing of XIP addition influence the magnitude and temporal dynamics of bacteriocin-associated activity. Methods A genetically modified *S. salivarius* strain was used to monitor the XIP-induced response associated with bacteriocin activity through a luminescent reporter. The effects of carbon source (glucose versus mannose), XIP concentration, timing of addition and progressive signal delivery were investigated. Luminescence emitted by the engineered strain was monitored over time to assess both the magnitude and temporal dynamics of the bacteriocin-associated response. A preliminary inhibition assay against *Lactococcus lactis* was also performed to assess the functional activity of the engineered strain.

Results

Compared to glucose, the addition of mannose to the medium increased both peak normalized luminescence and the integrated response over time, indicating that the metabolic environment modulates XIP-induced response. The increase of XIP concentration in the nanomolar range progressively enhanced both peak luminescence and the integrated response before reaching a plateau. A XIP concentration of 10 nM, close to saturation, was therefore selected for subsequent experiments. Early XIP addition resulted in a higher luminescence response than delayed addition, with the highest response magnitude observed when XIP was supplied at the onset of bacterial growth. Finally, progressive XIP delivery over 2h resulted in a more sustained luminescence response, compared to a single addition at growth onset. First inhibition assays showed that the engineered *S. salivarius* strain inhibited the growth of *L. lactis* under XIP-induced conditions, providing a first functional validation of bacteriocin-associated activity

Conclusions

These results demonstrate that XIP-dependent bacteriocin-associated activity can be externally tuned through both environmental and signal-related parameters. Importantly, beyond controlling the amount of XIP, adjusting the timing and temporal distribution of the signal altered the magnitude and duration of the response. Together with preliminary functional validation, these results suggest that synthetic XIP could provide a controllable means to modulate bacteriocinassociated activity and its dynamics in *S. salivarius* engineered strain.

Antimicrobial peptide biosensor for rapid bacterial identification: deciphering complex data using linear discriminant analysis

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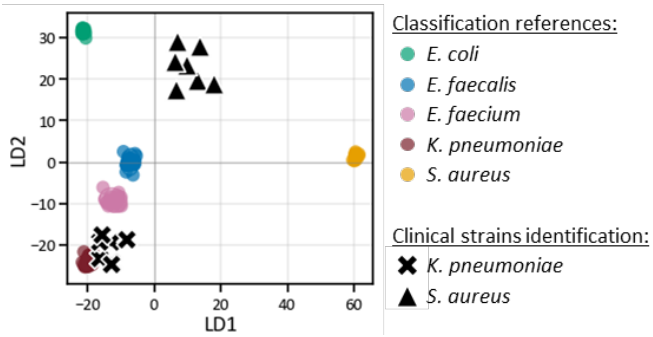
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More than 7.7 million deaths were associated with bacterial infections worldwide in 2019, including 2.9 million linked to bloodstream infections. Rapid diagnosis is essential to improve patient outcomes enabling appropriate antibiotic therapy to be initiated as early as possible. However, due to the low bacterial load in blood, standard diagnostic techniques require blood culture before bacteria can be identified. As a result, the first diagnostic information is generally available only after 24 h.

Using an antimicrobial peptide (AMP) micro-array, our aim is to develop a biosensor enabling bacterial identification directly during the enrichment phase. It relies on a surface plasmon resonance imaging (SPRi) platform that allow for the real-time monitoring of interactions between a probe, here the peptides, and an analyte, here the bacteria. Affinity profile recorded using SPRi were analysed by linear discriminant analysis (LDA) to enable unambiguous bacterial pathogen identification. This statistical method identifies the optimal linear combination of variables to maximize the separation between predefined groups. The arrays comprised 13 selected peptide sequences grafted through the C- or N-terminus, with each peptide–orientation combination replicated 7 times. Data were collected across three independent experiments and five bacterial strains. Due to the limited number of independent experiments, the responses from all seven replicate spots were used to train the LDA model. A leave-one-experiment-out validation strategy was used. For each validation iteration, one independent experiment was left out for testing, while the model was trained using all replicates from the remaining experiments. This procedure was repeated until each experiment had been used once as the test set. The LDA model was then applied to originally unidentified clinical strains.

The growth of *S. aureus, E. coli, K. pneumoniae, E. faecium*, and *E. faecalis* in tryptic soy broth was monitored and resulted in repeatable and distinct affinity profiles. Analyses were performed on samples spiked with a bacterial concentration ranging from 15 to 58 CFU/mL, thus comparable to that of the blood culture, and gave exploitable affinity profiles in less than 17 h. LDA analysis of these profiles enabled a successful bacterial classification with 93% accuracy. Only one experiment from the analysis of the *S. aureus* strain was misclassified. These results demonstrate that bacteria can be classified at the specie level. Clinical samples from patients of the Grenoble Alpes university hospital, originally unknown, were subsequently analysed with this biosensor. Although some misclassification remained, the LDA model was able to identify bacterial species despite the limited training dataset.



LDA analysis of the affinity profiles obtained with the AMP based biosensor

To conclude, we designed an AMP-based biosensor enabling rapid bacterial detection and identification at low concentrations within a single experimental step, followed by LDA-based data analysis. These results highlight the potential of statistical methods such as LDA to achieve reliable classification performance despite a limited experimental dataset. Overall, this device shows promise as a platform for the development of a rapid diagnostic tool.

Covalent immobilization of Ruminococcin C antimicrobial peptide on silicon surfaces for antibacterial application

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Biomaterial-associated infections remain a major challenge in biomedical field particularly due to bacterial adhesion and subsequent biofilm formation on implanted medical devices such as breast or knee implants. Surface functionalization with antimicrobial peptides (AMPs) represents a promising strategy to provide localized antibacterial activity while limiting the release of antimicrobial agents into the surrounding environment. In this study, we investigated the covalent immobilization of Ruminococcin C (RumC) derivate, an antimicrobial peptide, onto two clinically relevant materials – namely, silicon used in breast implants and titanium, a component of many bones protheses – with the aim of developing antibacterial surfaces.

The RumC peptide used in this study is not a natural isoform but rather a non-natural derivative named RC13. It features two modifications compared to the RumC5 sequence: A1W and T43K. The terminal lysine (T43K) was added to facilitate surface functionalization applications presented in this work. The peptide is produced via heterologous expression in *E. coli* following the protocol described in Chiumento et al., 2019, *Science Advances*, with a slight modification to the purification protocol. In solution, RumC and its derivatives appear to have no effect on Gram-negative bacteria (*E. coli* ATCC 25922), whereas for Gram-positive bacteria (*S. aureus* ATCC 25923), MIC values of approximately 12.5 µM were obtained.

A silane-based functionalization strategy was developed to enable the covalent immobilization of Ruminococcin C on both silicon and titanium substrates. Surface modification and peptide grafting were characterized and validated by X-Ray Photoelectron Spectroscopy (XPS and Polarization Modulation Reflection – Absorption InfraRed Spectroscopy (PM-RAIRS)). The antibacterial activity of the resulting functionalized surfaces was evaluated against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* (ATCC 25923), two representative Gram-negative and Gram-positive bacterial species. Microbioloigcal tests carried out on functionalized surfaces were confronted with bare substrates and towards initial bacterial innocum.

Characterization results show that the functionalization strategy using silane chemistry is effective, with 1.9 peptides immobilized per nm² for silicon and 0.9 peptides/nm² for titanium. This difference can be explained by the roughness of the silicon surface and the very dense APTES layer grafted before the peptide, compare to a more flat and homogeneous oxidized titanium surface.

RumC demonstrated 75% and 77% efficacy, respectively, against *E. coli* and *S. aureus* when comparing the functionalized silicon surface to the bare one. Concerning titanium, result showed no significant bactericidal effect against *E. coli*, whereas 67% efficacy is related for *S. aureus.*

Overall, these results support the covalent immobilization of AMPs as a promising strategy to develop antibacterial properties of biomaterial of clinical interest. The use of silane chemistry offers a versatile approach applicable on two different substrates, both soft and rigid. Surface immobilization allows localized antimicrobial activity at the interface between material and biological tissues. This strategy could contribute to the development of antimicrobial coatings designed to prevent bacterial colonization after medical device implantation. Finally, surface functionalization of inactive peptides in solution have proven to show a very good efficiency while combined in a complex 3 partners biointerfaces.

CLOSING LECTURE

Antifungal proteins from plants: Discovery, mechanisms of action and potential applications.

Marilyn Anderson

Trobe University, co-founded HEXIMA, Member of the Australia Academy of Science, Australia

Fungal infections remain one of the most neglected global health threats.[1] They affect an estimated 300million people worldwide every year and that burden is continuing to grow with rising resistance to all three classes of current antifungal drugs.[2] Global trade, use of antifungals in agriculture, travel and climate change are facilitating the spread of drug-resistant strains. Hence there is an urgent need for new antifungal drugs with different mechanisms of action. Plants have coevolved with fungi and have several strategies to avoid damage from fungal diseases. This talk will focus on the large and very diverse, plant defensin family and the different mechanisms they use to kill fungal pathogens. I will describe development of one of these defensins as a new class of antifungal drugs for clinical use up to the stage of Phase 2 trials.

1. UN news 30th June 2026
2. Blueprint for strengthening responses to fungal disease and antifungal resistance WHO 30th June 2026.

POSTERS

Unravelling the antimicrobial peptide resistance of *Zymomonas mobilis*

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Introduction:

Zymomonas mobilis is a facultatively anaerobic ethanologenic bacterium distinguished by exceptionally rapid fermentation, uncoupled energy metabolism, and recently discovered extreme resistance to antimicrobial peptides (AMPs). Its rapid Entner–Doudoroff pathway generates a high phosphorylation potential, while the respiratory system exhibits relatively low energy-coupling efficiency, resulting in substantial energy dissipation during growth [1-3]. As antimicrobial peptides primarily target bacterial cytoplasmic membranes, disrupt membrane integrity and energy coupling [4], the unusual bioenergetics of *Z. mobilis* may influence its susceptibility to these compounds. Understanding the basis of AMP resistance in this bacterium may provide insights into microbial adaptation and identify new opportunities for biotechnological applications

Objectives:

This study aimed to characterize the resistance of *Z. mobilis* to membrane-active AMPs and investigate the physiological basis of this phenotype. Furthermore, the contribution of membrane properties and the H^+-dependent F_1F_0-type ATPase to AMP resistance was examined.

Methods:

Six AMPs used in this study were published earlier, while five were novel peptides designed by the present project team [5]. Peptides were synthesized using solid-phase peptide synthesis (ProteoGenix, France). The MIC concentrations of AMPs were determined by quantification of pathogen growth in a 96-well plate microplate reader. ATP synthase activity was assessed by inducing an artificial transmembrane pH gradient and quantifying ATP production using the luciferin–luciferase assay. *Z. mobilis* knockout mutants were generated by homologous recombination. AMP–membrane interactions were further investigated using Langmuir–Blodgett compression isotherms of membrane lipid preparations.

Results:

Z. mobilis exhibited remarkably high resistance to the tested AMPs, substantially exceeding that of *E. coli* and other ESCAPE pathogens (Table 1). After the initial screening, further experiments were conducted using R10 (α-helix) and RP556 (antiparallel β-sheet) peptides. AMPs were shown to directly interfere with cytoplasmic membrane energy coupling, as concentrations above the inhibitory threshold largely eliminated pH gradient-induced ATP synthesis. Disruption of either the F_1 or F_0-ATPase subunit reduced resistance by approximately 2–3-fold (Figure 1.), demonstrating that cellular energy metabolism contributes to the resistance phenotype. Langmuir–Blodgett analysis revealed highly similar membrane lipid properties among the wild-type and mutant strains, indicating that the altered resistance was not primarily attributable to major changes in membrane lipid organization.

Table 1. Minimal inhibitory concentration (MIC) of AMPS against *Z. mobilis, E. coli* and ESCAPE pathogens.

	MIC, µg/mL						
Name	*Z. mobilis*	*E. coli*	*P. aeroginosa*	*K. pneumonia*	*E. faecium*	*S. aureus*	*C. acne*
RP556	250.0	4.0	2.0	4.0	2.0	1.0	2.0
LZ1	63.0	31.0	8.0	16.0	4.0	2.0	8.0
AA139	250.0	4.0	16.0	8.0	16.0	8.0	63.0
PA13	63.0	31.0	16.0	16.0	4.0	2.0	16.0
Oligo10	31.0	16.0	63.0	63.0	16.0	4.0	2.0
R1	63.0	8.0	8.0	4.0	16.0	8.0	2.0
R10	31.0	2.0	2.0	8.0	4.0	4.0	0.5
R11	250.0	125.0	63.0	63.0	16.0	16.0	0.3
R12	63.0	8.0	4.0	31.0	16.0	8.0	0.5
R13	63.0	4.0	4.0	8.0	16.0	4.0	0.3
R14	63.0	16.0	4.0	4.0	8.0	8.0	1.0

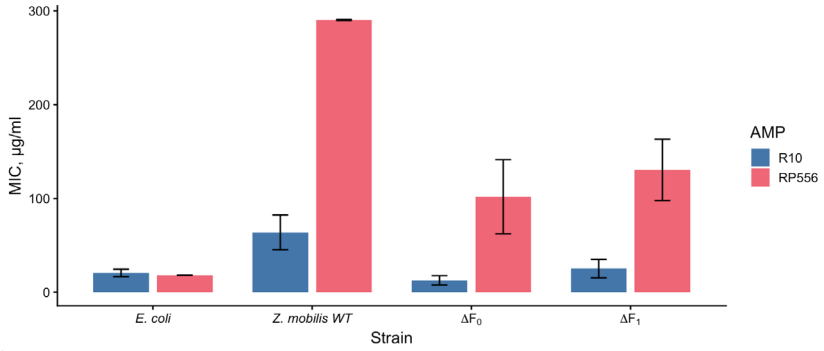


Figure 1. MIC of AMPs against E. coli and Z. mobilis WT, △F0 and △F1 knock-out mutants. Data represent mean values of 8 biological repeats □} standard deviation. Differences between the WT and E. coli, as well as WT and both mutant strains were statistically significant.

Conclusions:

Z. mobilis displays an exceptionally high resistance to membrane-active AMPs, representing a distinctive physiological phenotype. The findings demonstrate that resistance is strongly associated with the bacterium's unusual energy metabolism and its ability to tolerate disruption of membrane energy coupling. The contribution of the H_+-dependent F_1F_0-type ATPase further supports a role for membrane energization in maintaining resistance. This phenotype may provide *Z. mobilis* with an ecological advantage in its possibly AMP-rich native environments and highlights its potential as a host for recombinant AMP production.

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Biaryl peptide macrocyclization by GpeC for antibacterial discovery

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Introduction

Biaryl-crosslinked macrocyclic peptides are promising scaffolds for antibacterial discovery. However, their synthesis remains constrained by substrate-dependent cyclization strategies, limiting access to diverse analogues for biological screening. Ribosomally synthesized and post-translationally modified peptides (RiPPs) offer a source of enzymes for expanding this chemical space.

Objectives

We aimed to identify and characterize a broadly tolerant P450 macrocyclase for peptide biaryl cyclization and explore the antibacterial potential of the resulting peptide pools.

Methods

Genome mining guided the identification of GpeC, which was characterized through in vitro enzymatic assays, high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy. X-ray crystallography, molecular dynamics simulations and site-directed mutagenesis were used to investigate substrate recognition. Systematic substitutions assessed tolerance at the two internal positions of the tetrapeptide core. Peptide pools were screened against Micrococcus luteus by monitoring inhibition-zone formation.

Results

GpeC catalyzed biaryl macrocyclization within tetrapeptide cores embedded in leaderless nine-residue substrates. Beyond native Trp-Tyr C-C coupling, it supported alternative aromatic pairings with C-O and C-C/C-N linkages. The 2.62 Å crystal structure, together with computational and mutational analyses, supported an adaptive substrate-recognition mechanism. Substitution screening showed tolerance for 19 of 20 canonical amino acids at each internal position, with proline excluded. Diverse noncanonical residues were also accepted, providing opportunities for scaffold diversification. Chemoenzymatic synthesis of the biaryl-cyclized core of rubrin further demonstrated synthetic utility [1]. In preliminary antibacterial screening, 11 of 17 evaluable peptide pools produced inhibition zones against Micrococcus luteus. These results support antibacterial potential at the pool level and identify active pools for further characterization.

Conclusions

GpeC provides a versatile biocatalytic route to diverse biaryl peptide macrocycles for antibacterial discovery. The initial screening results warrant deconvolution of active pools to identify individual active peptides, followed by broth-based confirmation and quantitative assessment of antibacterial potency.

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Targeted Screening of Human Skin Commensal Staphylococci Identifies a Novel Coagulase-negative Strain Producing Potent Anti-MRSA Activity

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Introduction & Objectives:

Human skin-colonizing coagulase-negative *Staphylococci* (CoNS) mediate colonization resistance against opportunistic pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA). This study aimed to perform targeted screening across a broad panel of skin commensal isolates to identify functional antibacterial strains, delineate the kinetics of active substance secretion relative to growth phases, and evaluate the bio-efficacy of refined bacterial supernatant extract regarding antibacterial spectrum, dynamic growth inhibition, and sustained antibiofilm activity.

Methods:

Skin swabs collected from subjects with skin dermatitis yielded a total of 548 staphylococcal isolates, comprising 105 coagulase-positive (CoPS) and 443 coagulase-negative (CoNS) strains. All CoNS were evaluated for antagonistic activity against clinical MRSA strains using disk/well diffusion assays. Secretion dynamics of the active isolate were monitored by co-assessing growth (OD_{600}) and time-course antibacterial activity. Active substances were recovered via 1-butanol extraction from cell-free supernatant. The extract's bio-efficacy was evaluated through Grampositive spectrum profiling, CFU enumeration for 24-h anti-MRSA growth, and 72-h antibiofilm microplate assays quantified by 0.5% crystal violet staining.

Results:

Primary screening of all isolated CoNS revealed 4 candidate strains with anti-MRSA activity, among which only one strain, CUHK_CoNS185, exhibited consistent and notable activity. Accumulation of active antibacterial substances by CUHK_CoNS185 was strictly growth-dependent, attaining maximum potency during early stationary phase. The 25% (v/v) 1-butanol extract (BE) displayed a broad antibacterial spectrum against multiple Gram-positive pathogens, including reference ATCC strains of *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus agalactiae, Streptococcus pyogenes, Enterococcus casselliflavus, and Enterococcus faecalis*. In 24-h growth assays, the 25%(v/v) extract achieved up to 4-log reductions in MRSA CFU/mL across clinical strains (including MRSA ST22, ST30, ST45, ST59, ST239, and ST8) compared to the vehicle growth control (Two-way ANOVA, $p<0.001$). Microtiter assays demonstrated sustained antibiofilm efficacy, maintaining >85–95% biofilm inhibition across all test strains over 24, 48, and 72 h.

Conclusion:

Screening of commensal skin isolates yielded a novel CoNS strain capable of secreting potent antibacterial substances during early stationary growth. The resulting extract exhibits significant growth suppression and sustained antibiofilm activity against MRSA, underscoring its potential as a microbiome-derived therapeutic agent.

Exploring *Bacillus*-Derived Antimicrobial Peptides for Sustainable Plant Disease Management: A Literature-Based Perspective

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Plant diseases constrain agricultural productivity and threaten food security, highlighting the need for effective and environmentally compatible disease management strategies. Dependence on conventional antibiotics and synthetic pesticides has encouraged interest in alternative antimicrobial agents with diverse biological activities. Antimicrobial peptides (AMPs) represent promising candidates because their structural and functional diversity offers opportunities for targeting different pathogens. Among microbial sources, *Bacillus* species have attracted attention for their capacity to produce biologically active peptides. This abstract summarises a published investigation that integrated genome screening, deep learning, molecular characterisation, and experimental validation to discover *Bacillus*-derived AMPs with potential relevance to agricultural disease management. More than 6,700 *Bacillus* genomes were analysed to identify short peptide sequences comprising 10–100 amino acids. Four deep learning architectures, BERT, Mamba, CNN–LSTM, and CNN–Attention, were applied to predict antimicrobial potential. Sequences predicted positively by all four models were retained, yielding 4,993,389 putative AMPs. Subsequently, 10,000 randomly selected candidates were assessed using additional prediction tools; 23 sequences were consistently classified as AMPs by every tool. Two candidates, designated cAMP_1 and cAMP_2, were prioritised based on their physicochemical properties and predicted structural characteristics. Molecular dynamics simulations supported their selection for experimental evaluation. Against *Escherichia coli*, minimum inhibitory concentrations were 7.813 µg/mL for cAMP_1 and 62.5 µg/mL for cAMP_2. Both peptides exhibited a minimum inhibitory concentration of 62.5 µg/mL against *Staphylococcus aureus*. They also inhibited bacterial pathogens responsible for tobacco angular leaf spot, bacterial wilt, and wildfire disease. Both candidates inhibited *Fusarium oxysporum* mycelial growth at 2 and 4 mg/mL and the oomycete *Phytophthora nicotianae* at 4 mg/mL.These findings illustrated how computational screening could reduce a large sequence collection to a limited set of candidates for laboratory investigation. Importantly, the predicted sequences represented a discovery pool rather than experimentally confirmed antimicrobial agents. The subsequent assays supplied evidence of activity for the selected peptides, demonstrating why laboratory validation remains essential when interpreting computational predictions. Differences in inhibitory concentrations across test organisms also indicated that antimicrobial performance depended on the peptide and target organism. Consequently, evidence of activity against one microorganism should not automatically be interpreted as equivalent effectiveness against other pathogens. For agricultural development, the observed laboratory activity provides a basis for further investigation rather than proof of disease control under cultivation conditions. Peptide stability, production feasibility, formulation, delivery, and persistence require assessment before practical application. Crop compatibility and effects on beneficial microorganisms should also be examined to determine whether antimicrobial activity can be translated into useful and selective disease suppression. Greenhouse experiments and subsequent field evaluations would help establish performance within complex plant-associated environments. Overall, the published investigation demonstrated a complementary relationship between genome-guided prediction and experimental testing. Its principal contribution was the identification of two experimentally active candidates from a substantially larger computational search space. Further optimisation and agricultural validation could clarify their suitability as components of integrated plant disease management. The approach offers a foundation for discovering microbial peptides while maintaining a distinction between predicted antimicrobial potential, demonstrated laboratory activity, and confirmed effectiveness in agricultural production systems.

Heterologous expression of the novel recombinant defensin CIDEF1 from *Cryptocaryon irritans* in *Chlamydomonas reinhardtii*: antimicrobial potential and biotechnological applications

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Introduction:

The dramatic increase in multi-resistant microorganisms highlights the critical need for novel and efficient antimicrobial agents. Cysteine-stabilized αβ (CS-αβ) defensins represent a promising class of antimicrobial peptides (AMPs) due to their broad efficacy against pathogens. In this context, the green microalga *Chlamydomonas reinhardtii* stands out as an ideal, sustainable biofactory, combining rapid growth, absence of endotoxins, GRAS (Generally Recognized as Safe) status, and the capacity for complex post-translational modifications required for the proper folding of disulfide-rich proteins.

Objectives:

To identify in silico a novel defensin-like peptide (CIDEF1) from the parasitic ciliate *Cryptocaryon irritans*, achieve heterologous expression in the nucleus of *C. reinhardtii* (strain UVM4), and perform structural and preliminary functional characterizations.

Methods:

CIDEF1 identification was accomplished through a bioinformatic pipeline screening ciliate genome. Structural characterization involved comparative modeling, molecular dynamics (MD) simulations, and molecular docking with membrane phosphoinositides. For nuclear expression, the codon-optimized CIDEF1 sequence, containing an N-terminal signal peptide for translocation and a C-terminal FLAG tag, was cloned into the pJP32 vector under the control of the chimeric HSP70A-RBCS2 promoter and RBCS2 3' UTR, harboring the *ble* gene conferring zeocin resistance. Transformation into *C. reinhardtii* UVM4 was performed via glass beads. Transformants were verified by colony PCR screening and selected on zeocin-supplemented media. Protein expression and accumulation were evaluated via anti-FLAG Dot Blot and Western Blot immunoassays.

Results:

In silico analyses revealed that CIDEF1 exhibits a canonical CS-αβ defensin scaffold stabilized by three conserved disulfide bridges. MD simulations (200 ns) confirmed high conformational stability, maintaining a low average backbone RMSD (< 0.25 nm) and stable radius of gyration. Molecular docking demonstrated strong binding affinity to PIP2 (-8.5 kcal/mol), driven by electrostatic interactions at the cationic surface, suggesting targeted fungal membrane anchorage. Experimentally, transformation of the UVM4 strain yielded stable transformants under selective media. Transgene integration was confirmed by colony PCR, amplified with specific primers for the CIDEF1-FLAG cassette. Dot Blot immunoassays using anti-FLAG antibodies displayed distinct signal intensity in transformed line extracts compared to wild-type controls, validating the accumulation of recombinant CIDEF1 in the microalgal host.

Conclusions:

C. reinhardtii proved to be a viable and efficient host platform for expressing the novel peptide CIDEF1. Positive colony PCR and Dot Blot assays validate transgene integration and protein production, underscoring the potential of ciliated protists as sources of bioactive molecules and microalgae as green biofactories for antimicrobial production. Further antimicrobial assays and application tests in functional bio-based food packaging are ongoing.combination was further evaluated in a *S. agalactiae* challenge model in Nile tilapia (*Oreochromis niloticus*) with or without heat stress, and in a field trial involving dairy cattle during the dry period by monitoring somatic cell count (SCC).

Results

All four concatemeric bacteriocins retained antimicrobial activity following recombinant production and purification. EntPx4 displayed MICs of 1.25 μM against all reference pathogens, whereas AurA53x4 inhibited S. agalactiae at 1.25 μM and MSSA and MRSA at 2.50 μM. Although FICI analysis indicated indifferent interactions between AurA53x4 and EntPx4, their combination showed a more favourable profile in bactericidal assays. High killing efficiencies were maintained across several combinations, with bactericidal activity reaching >95% at combined total concentrations of 0.31 μM for S. agalactiae, 0.63 μM for MSSA and 2.66 μM

for MRSA in selected pathogen-specific conditions. A general dose-sparing effect was observed upon combination of EntPx4 and AurA53x4. In vivo, prophylactic administration of the AurA53x4-EntPx4 combination resulted in 75% survival following challenge with a clinical S. agalactiae isolate in tilapia, while antibiotic supplementation resulted in <5% of tilapia survival. In the ruminant field trial, bacteriocin treatment was associated with a 35.7% reduction in somatic cell count (SCC), compared to a 8.3% reduction observed upon antibiotic treatment.

Conclusions

This work demonstrates the potential of concatemerization as a versatile bacteriocin engineering strategy for the development of novel antimicrobial interventions based on class II bacteriocins for One Health solutions. The combination of AurA53x4 and EntPx4 generated promising antimicrobial activity in vitro and showed encouraging results in both aquaculture and livestock settings.

Characterization of Actifensin variants encoded in Actinomyces through heterologous expression in yeast *Komagataella phaffii* (*Pichia pastoris*)

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Introduction

The need for additional treatment options for pathogenic bacterial infections, including recurrent *Clostridioides difficile* infection, motivated this investigation of alternative antimicrobial treatments. Actifensin, originally identified from Actinomyces ruminicola DPC7226, belongs to a diverse family of mainly Actinomyces-associated defensin-like bacteriocins whose natural variants can vary by over 52% mean amino acid identity. This diversity provides a basis for exploring peptides with distinct antimicrobial properties.

Objectives

To produce and purify several phylogenetically-different natural actifensin variants, assess their antimicrobial activity, and establish a foundation for future evaluation against clinically-relevant pathogens, including *C. difficile*. Previous work established some preliminary activity against *C. difficile* along with other indicators.

Methods

Seven natural actifensin variants were selected from a potential of 49 encoded based on sequence dissimilarity and phylogenetic distance from one another to capture the diversity within this novel peptide family. Genes encoding the mature peptides were cloned into the pPICZαA and expressed in *Komagataella phaffii* (syn. Pichia pastoris) X-33 under the methanol inducible AOX1 promoter. Small-scale 125 ml broths of transformants with known activity were screened for activity after X days via well diffusion assay[IS1.1]. Cell-free culture supernatants were then screened against *Lactobacillus delbrueckii* subsp. bulgaricus LMG6901, with the untransformed K. phaffii X-33 and K. phaffii YIS001 producing archetypal actifensin A from *Actinomyces ruminicola* DPC7226 serving as negative and positive controls, respectively. Peptides were recovered using XAD adsorption, C18 solid-phase extraction, and HPLC fractionation. Selected variants were analysed by MALDI-TOF MS. Minimum inhibitory concentrations (MICs) were determined for YIS181 and afnA against selected bacterial indicators.

Results

Seven variants were successfully produced and purified: YIS181 (CCUG34286_4), YIS149 (HMSC06D04), YIS188 (F0588), YIS117 (2119_4), YIS132 (2119_4), YIS164 (Pn6N), and YIS203 (Def 1). Cell-free supernatants from all seven variant-producing strains inhibited *L. bulgaricus LMG6901*. YIS164 produced the smallest inhibition zone in the cell-free supernatant screen, but antimicrobial activity remained detectable during purification. The positive control exhibited inhibition, whereas the negative control showed no inhibitory activity. MALDI-TOF MS analysis of selected variants yielded masses consistent with those predicted. YIS181 exhibited MICs of 1 µg/mL against *L. bulgaricus LMG6901* and 0.25 µg/mL against *Micrococcus luteus* APC4061. For AfnA, MICs were 8 µg/mL against *Streptococcus pneumoniae* DSM1 14377, 4 µg/mL against *Streptococcus agalactiae* APC 1055, and 32 µg/mL against both *Listeria monocytogenes* EDG-e and the vancomycin-resistant *Enterococcus faecium* VRE6.

Conclusion

Seven actifensin variants were produced and purified, with antimicrobial activity detected in all corresponding culture supernatants. MIC measurements demonstrated YIS181 activity against two indicators and afnA activity against several clinically relevant bacteria. These findings support further comparative characterisation of the peptide family. Future evaluation against more clinically-relevant pathogens such as *C. difficile*, together with assessment of stability, and effects on beneficial microbiota, will help establish its potential for applications in human infection treatment and management. This work could allow for the larger scale production of antimicrobial peptides for clinical treatment of pathogenic infections.

Lasso Peptides against Tuberculosis – from Genome Mining to Activity Analyses

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Introduction

With an estimated 10.7 million active cases and 1.23 million deaths in 2024, tuberculosis (TB) is the deadliest infectious disease in the world.1 TB is caused by mycobacteria, and different treatment options are available. However, the treatment is lengthy and intensive, and suboptimal or uncontrolled therapy can foster the development of drug-resistant strains.1 This highlights the urgent need for new agents against TB. Naturally occurring peptides, including ribosomally synthesized and post-translationally modified peptides (RiPPs) are a promising source for new drugs.2 Lasso peptides are an ascending class of RiPPs that contain 13–33 amino acids and a characteristic isopeptide bond forming an N-terminal macrolactam ring.3 The C-terminal tail is threaded through this macrolactam ring, resulting in a slip knot shape that makes these peptides highly stable against heat, acids, and proteases, refuting one of the main disadvantages of peptide drugs.

Objectives

The main objective of this project is the characterization of antimycobacterial lasso peptides. This includes the identification of uncharacterized bacteria harboring promising biosynthetic gene clusters (BGCs), the (heterologous) expression of these BGCs, and the isolation of produced lasso peptides, as well as the analysis of their bioactivity and specificity.

Methods

We combine bioinformatic tools for genome mining with molecular biological and biochemical approaches to generate expression systems and protein purification procedures. In addition, chemical techniques are used for extraction and analysis of the lasso peptides.

Results

Two lasso peptides with antimycobacterial activity and unusual C-terminal methylations – lassomycin4 and kitamycobactin5 – have been reported in the literature. In our study, we identified several actinobacteria encoding BGCs that are homologous to the proposed BGCs of lassomycin and kitamycobactin through extensive genome mining. Production of lasso peptides in their native hosts is often limited; therefore, we aimed to establish a heterologous expression system for our potential antimycobacterial lasso peptides in Streptomyces hosts. Different approaches were applied to produce the lasso peptides, including investigating different Streptomyces strains, testing various promoter systems, and adjusting cultivation conditions. Following cultivation and production, the lasso peptides were extracted and analyzed using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). These HPLC-MS/MS data revealed the successful production of several lasso peptides. Their antimycobacterial activity was confirmed by activity assays against Mycobacterium phlei and M. smegmatis. Currently, we are purifying the produced lasso peptides via HPLC and investigating the role of their unusual C-terminal methylation.

Conclusions

In summary, we used genome mining to identify several potential producers of antimycobacterial lasso peptides. While the heterologous production and antimycobacterial activity of the selected lasso peptides seem promising, their purification is challenging and the role of the C-terminal methylation remains elusive. Thus, these two subprojects are currently under investigation. In future, we plan to produce and purify more antimycobacterial lasso peptides, investigate structure-activity relationships, and analyze their mycobacterial specificity through testing against other bacteria and mammalian cells. We aim to use these finding to advance the development of lasso peptides as antimycobacterial drugs, thereby helping to fight TB, the world's deadliest infectious disease.

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From precursor to potent antibiotic: investigating the maturation of darobactins targeting Gram-negative pathogens

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Introduction:

Darobactins are a family of bicyclic ribosomally synthesized and post-translationally modified peptides (RiPPs) with potent antibacterial activity against Gram-negative pathogens. They target BamA, an essential protein involved in outer membrane biogenesis.[1-2] Through biosynthetic engineering, several derivatives, including darobactin 22 (D22), have been developed with enhanced antibacterial activity against clinically relevant pathogens.[3–5] Despite significant progress in understanding darobactin biosynthesis, the final maturation step remains poorly understood. While the radical SAM enzyme DarE catalyzes the bicyclization of the precursor peptide, previous studies have demonstrated that endogenous enzymes in *Escherichia coli* (*E. coli)* can process the modified precursor into mature darobactin.[6] However, the enzymes responsible for this proteolytic processing remain unidentified.

Objectives:

This work aims to better understand darobactin maturation by investigating the proteolytic processing of its precursor peptide and identifying potential host-encoded enzymes involved in this final biosynthetic step.

Methods:

Several complementary approaches were combined to investigate darobactin precursor processing, including transcriptomic and proteomic analyses of darobactin-producing *E. coli*, screening of single-gene knockout strains, comparative genomics, and *in vitro* cleavage assays using bacterial lysates. Based on these investigations, selected candidate proteins were heterologously expressed and purified for further biochemical characterization. In parallel, bicyclized D22 precursor was produced using an approach adapted from Guo *et al*.[6] and purified to serve as a substrate for *in vitro* assays.

Results:

Combining genetic, proteomic and biochemical approaches allowed us to narrow down the selection of potential enzymes involved in darobactin maturation. *In vitro* experiments with bacterial extracts provided evidence of precursor processing activity, supporting further investigation of host-encoded proteases. Selected candidate proteins have now been successfully expressed and purified. Further assays using cyclized D22 precursor are being prepared to investigate their individual and combined activities and potential roles in the maturation process.

Conclusions:

Combining complementary screening approaches with *in vitro* biochemical characterization provides a foundation for identifying the enzymes involved in darobactin precursor processing. Understanding this final maturation step will provide valuable insights for overcoming current biosynthetic limitations and optimizing heterologous darobactins production.

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Environmental change and pathogen susceptibility: decoding mussel immunity against *Francisella halioticida* through antimicrobial peptides

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Introduction:

With climate change, marine animals are increasingly exposed to emerging pathogens. *Francisella halioticida* has been associated with high mortalities in abalone, Yesso scallop and mussels in Europe. Molluscs rely solely on innate immunity, antimicrobial peptides (AMPs) act as key immune effectors. Although AMPs are highly diverse across the genus Mytilus, current research mainly focuses on M. galloprovincialis and M. coruscus, leaving natural hybrids largely unexplored.

Objectives:

Characterize the AMP repertoire of natural hybrid mussels *Mytilus sp.* farmed in Normandy (France) and understand the response after challenge with a low-virulence (FR21) or a high-virulence (FR22c) strain of *F. halioticida*.

Methods:

Mussels were injected with FR21, FR22c (~2 × 10^7 CFU/mL) or sterile broth, and gills were sampled at 24 h and 72 h post-injection (five pools of five mussels per condition). Known AMP families were predicted from a *de novo* gill transcriptome (Illumina NovaSeq) using conserved cysteine motifs in the software PepTraq. Predictions were validated by peptidomics (nanoLC-TIMS-TOF MS/MS). Differential expression was analysed at the family level with Salmon/DESeq2 ($p < 0.05$, $|\log2FC| > 1$). Novel candidate AMPs were predicted among differentially expressed transcripts with strict selection criteria (a precursor length under 150 amino acids, the presence of an N-terminal signal peptide, and MS/MS mass spectrometry validation) and submitted to CAMP and AmpInR prediction tools.

Results:

In total, 82 AMP sequences were predicted, covering all known *Mytilus* families except mytichitin and crustin-like. Linear myticalins were the most diverse, and most sequences were proteoforms of known AMPs. MS/MS confirmed eight families or subfamilies and refined mature peptide predictions. At 24 h, both strains induced the same profile, downregulation of myticins A, B and C and mytilins (B, D), while only a mytimacin-like peptide was upregulated. At 72 h, responses diverged with virulence, FR21 induced myticin C and mytimacin 1, whereas FR22c induced myticalin A and mytimycin. This study proposed a novel potential linear anionic AMP rich in Asp, Gly and Lys confirmed by MS/MS, named Mytidin. Homologues have been found in the genome of other *Mytilus* species.

Conclusions:

The mussels used in this study expressed a broad, polymorphic AMP repertoire. *F. halioticida* triggered an early, virulence-independent repression of canonical AMPs in the gills. This was followed by virulence-associated responses, classical effectors (myticins, mytimacins) against the low-virulence strain, and myticalins and mytimycin against the high-virulence strain. Mytidin extends mussel AMPs beyond cysteine-rich and cationic families. Functional assays against *F. halioticida* are now needed to clarify how these peptides contribute to disease outcome and to mussel resilience against emerging pathogens.

Investigating Evolutionary Mechanisms and Classifier Performame in Class II Bacteriocins

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Bacteriocins are ribosomally synthesised antimicrobial peptides (AMPs) produced by bacteria. These naturally occurring AMPs have the potential to be used alongside conventional antibiotics. Machine learning (ML) classification allows for the identification of novel class II bacteriocins from amino acid sequences, enabling their detection and characterisation across global microbiomes. However, their small size, structural diversity, and underrepresentation in ML training sets make accurate in silico prediction challenging. For instance, class II bacteriocins are produced either as leaderless peptides, requiring no cleavage, or as precursor proteins containing a leader peptide that is removed during maturation. In some bacteriocins, the leader peptide functions as a signal peptide, a short N-terminal extension that directs the precursor for secretion. In silico retention of these signal peptides, may therefore substantially compromise the accuracy of computationally identifying bacteriocins.

We evaluate the accuracy of existing AMP ML classifiers and the effect of removing detected signal peptides from sequences prior to classification. These findings will help ensure the accurate classification of class II bacteriocins, and support investigations into their evolutionary mechanisms, including horizontal gene transfer and potential *de novo* emergence.

We created a large database by mining all available genomes and metagenomes from GenBank and MGnify for small ORFs (smORFs). In this concatenated database of unique smORFs, signal peptide prediction was run along with AMP classifiers. Simultaneously, we evaluated AMP classifiers, utilising a database of species-specific orphan genes (SSOGs) from the human gut microbiome. Five AMP classifiers (amPEPpy, AMPlify, cAMP, EvoGradient and Macrel) were run on both the precursor amino acid sequences and sequences with the signal peptide cleaved, if present. Following this, expression data from a recently released paper on the same SSOG dataset was analysed to validate candidate AMPs (c-AMPs) on a transcriptomic level.

The concatenated database contains 133,382,552 unique smORFS, of these, 10,421,911 contain a predicted signal peptide. With regards to the SSOG dataset, significantly more c-AMPs were predicted when the signal peptide was removed, compared to retained. Interestingly, classifiers showed little agreement on predicted peptides, with no single c-AMP identified by all 5 of them. Furthermore, expression level validation appears to generally be higher on the candidates whose signal peptide has been cleaved. For instance, in amPEPpy, 95.1% of the c-AMPs predicted when the signal peptide is removed exhibited detectable expression, compared to 39.3% when the signal peptide is retained.

These results illustrate that some orphan genes may encode entirely novel AMPs, but their identification may be challenging. This emphasises the importance of continually improving bacteriocin identification pipelines. This includes the removal of signal peptides from input sequences, which has previously been overlooked, thereby leading to potentially biased results. Supplemental research is ongoing to further elucidate the discrepancies between classifiers and determine which perform more accurately for the specific task of identifying novel bacterial AMPs.

Isothermal microcalorimetric analysis of the antibacterial and antibiofilm activity of ε-poly(L-lysine)

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Chronic wound infections remain a major clinical challenge due to biofilm formation and antibiotic resistance, highlighting the need for alternative antimicrobial strategies and reliable susceptibility testing methods. Exposure to antimicrobial peptides can alter bacterial metabolism and associated heat production [1]. Isothermal microcalorimetry (IMC) is a nondestructive technique that enables real-time monitoring of the heat flow associated with bacterial metabolic activity. Requiring no repeated sampling during measurement, IMC is particularly well suited for studying antimicrobial activity in biofilm-embedded cells without disrupting the biofilm structure [2]**.** ε-poly(L-lysine) (ε-PLL) is a naturally occurring cationic homopolypeptide with promising antimicrobial and antibiofilm activity [3], [4].

Here, we assessed the antibacterial activity of ε-PLL against wound-associated bacteria*, Staphylococcus aureus* and *Pseudomonas aeruginosa,* under protein-rich, wound-like conditions. We further evaluated IMC as a real-time complement to conventional antimicrobial susceptibility testing for ε-PLL, using metabolic heat production to determine heat-based inhibitory concentration in planktonic bacteria and characterize antimicrobial effects on biofilm-associated cells.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined in Mueller–Hinton broth (MHB), with or without 50% (v/v) simulated wound fluid by using optical density measurements. IMC was then used to monitor heat-flow kinetics in planktonic culture and 24h-old *P. aeruginosa* biofilms exposed to increasing ε-PLL concentrations. Antimicrobial activity was assessed based on the delay and reduction of heat-flow peaks compared with untreated growth controls. The minimum heat inhibiting concentration at 90% ($MHIC_{90}$) was defined as the lowest ε-PLL concentration reducing metabolic heat production by at least 90% relative to untreated planktonic controls.

ε-PLL showed MICs and MBCs of 15–31 µg/mL in MHB against both *S. aureus* and *P. aeruginosa*. Antibacterial activity was maintained in the presence of 50% simulated wound fluid. IMC revealed clear concentration-dependent inhibition of planktonic bacterial metabolism following ε-PLL exposure, with MHIC90 values of 4.8 µg/mL for *S. aureus* and 10 µg/mL for *P. aeruginosa*. These values were within three-fold of MICs measured by conventional broth microdilution*.* Notably, MHIC90 values were below the corresponding MIC range, highlighting the sensitivity of IMC to ε-PLL-induced metabolic inhibition at concentrations insufficient to prevent visible bacterial growth. The IMC heat flow patterns at inhibitory and subinhibitory concentrations also provided additional insights into the nature of inhibition. In 24-h-old *P. aeruginosa* biofilms, ε-PLL induced distinct concentration-dependent alterations in heat-flow profiles, including delayed heat production, reduced peak intensity, and prolonged time to peak. These changes were observed at concentrations >62.5 µg/mL, above the planktonic MIC range.

Overall, ε-PLL retained rapid and potent bactericidal activity against *S. aureus* and *P. aeruginosa* in a wound-like environment. IMC provided real-time characterization of ε-PLL-induced changes in bacterial metabolic activity in both planktonic and biofilm states, supporting its potential as a highly sensitive and less time-consuming complementary approach to antimicrobial susceptibility testing. These results provide the rationale for further evaluation of ε-PLL-loaded hydrogels as the next generation of antimicrobial wound dressings for the prevention and treatment of biofilm-associated chronic wound infections. These formulations are currently being evaluated using IMC.

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Optimization of antimicrobial peptide fragments derived from *Cereus sp.*

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The growing threat of antimicrobial resistance (AMR) has intensified the search for antibiotic alternatives, with antimicrobial peptides (AMPs) emerging as promising candidates due to broad-spectrum activity and low resistance-inducing potential. This study aimed to identify, optimize, and rank AMP fragments derived from *Cereus* sp. using an integrated *in silico* pipeline, prioritizing candidates with predicted broad-spectrum efficacy against clinically relevant pathogens while preserving beneficial gut commensals. Four long peptides (70–90 aa; three snakins, one defensin) were computationally fragmented using CAMPr4 into 10-, 15-, and 20-aa fragments. Fragments were filtered with Antimicrobial Peptide Scanner v2 (AMP probability >0.75), then screened with APEX to predict minimum inhibitory concentration (MIC) against *E. coli* ATCC 11775. The two top fragments were used as templates for alanine-scanning optimization; the best variant underwent a second round involving a two-residue extension, followed by screening against a broader pathogen panel. Final ranking combined predicted MIC against *E. coli* ATCC 11775 and *P. aeruginosa* PAO1 with MIC against gut commensals (*Roseburia obeum*, *E. coli* Nissle, *Clostridium symbiosum*), favoring low pathogen MIC and high commensal MIC. CAMPr4 yielded 588 fragments (211 of 10 aa, 196 of 15 aa, 181 of 20 aa). AMP Scanner v2 flagged 60 15-aa and 98 20-aa fragments above threshold, favoring longer fragments. APEX identified 16 fragments with predicted MIC <100 µg/mL against *E. coli* ATCC 11775 (1 from CF267, 8 from CF15, 7 from CH167), with CF15 as the most promising source. CF15-4155 (62.71 µg/mL) and CF15-4357 (55.75 µg/mL) showed the strongest activity and were selected for optimization. Alanine scanning generated 15 variants per template, with CF15-4155(N8) showing the greatest improvement. The second optimization round produced 37,905 sequences, with predicted MICs from 133.31 to 16.27 µg/mL; ~16% (5,947) had MIC <50 µg/mL. Combined screening against *E. coli* ATCC 11775 and *P. aeruginosa* PAO1 identified 11 top candidates, all derived from CF15-4155. Cross-referencing commensal MICs narrowed these to four fragments: R2K4A8, K2R4A8, R2R4A8, and K2R6A8. AlphaFold 3 structures showed R2R4A8, K2R6A8, and K2R4A8 structurally similar to WT, while R2K4A8 showed slight deviation. K2R4A8 displayed the most favorable overall profile: MICs of 20.42 µg/mL (*E. coli* ATCC 11775), 49.92 µg/mL (*P. aeruginosa* PAO1), 124.85 µg/mL (*C. symbiosum*), 164.02 µg/mL (*R. obeum*), and 710.66 µg/mL (*E. coli Nissle*), up to ~35-fold higher against commensals than pathogens. These findings demonstrate the value of integrating computational fragmentation, machine learning-based screening, and iterative optimization to accelerate discovery of selective, broad-spectrum AMPs against AMR while minimizing off-target disruption of the gut microbiota.

Screening Human Microbiome Derived Short Peptides Targeting Sortase A for Antivirulence Discovery Using a PPI Affinity Prediction Model

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Introduction

Antimicrobial resistance has eroded conventional antibiotic efficacy, highlighting the need for strategies that interfere with bacterial virulence. Sortase A (SrtA) is a membrane-associated transpeptidase in *Staphylococcus aureus* whose extracellular catalytic domain recognizes LPXTG motifs and anchors surface proteins to the cell wall. These proteins contribute to adhesion and infection, and genetic disruption of SrtA attenuates virulence. Its natural peptide recognition and extracellular accessibility make SrtA an attractive target for peptide discovery. We hypothesize that human-associated microbial peptide repertoires contain potential SrtA ligands and that affinity prediction can prioritize this sequence space for experimental screening.

Objectives

We aim to apply a protein–protein interaction (PPI) affinity prediction framework to prioritize human microbiome-derived short peptides that bind *S. aureus* SrtA for synthesis, binding measurements and evaluation of potential antivirulence activity.

Methods

A retrospective SrtA benchmark was assembled from 19 published peptide binding records spanning four literature series. The prediction framework uses pretrained protein language model representations and structural interface information to score protein–peptide interactions. SaProt-based and pure-v7 affinity scores were combined at a 1:2 ratio, and predictions were compared with experimental affinity labels using Pearson and Spearman correlations. The framework was then applied to human microbiome-derived peptide candidates from oral/saliva and mixed skin/respiratory screening batches, alongside an earlier batch. Predicted complexes underwent structural confidence filtering and pose refinement. Model ranking prioritized candidates for molecular dynamics simulations, followed by endpoint rescoring and pooled selection across the three batches.

Results

In the retrospective SrtA benchmark, the combined score achieved a Pearson correlation of 0.757 and a Spearman correlation of 0.812 with experimental affinity labels. The predicted median ranking matched the measured order of all four literature series, supporting prioritization across peptide series. The screening workflow produced shortlisted candidates, from which 20 peptides of 13–20 amino acids were selected by mean endpoint confidence. These are computational candidates awaiting experimental validation. Planned experiments include MST binding measurements, SPR confirmation and FRET-based SrtA activity assays, followed by bacterial adhesion and surface-protein anchoring assays. Growth measurements will distinguish antivirulence effects from direct antibacterial activity.

Conclusions

Integrating affinity prediction with human microbiome mining provides a route to prioritize peptides for SrtA-directed discovery. The retrospective results support model-assisted screening, while binding and functional assays will determine whether the selected candidates act as SrtA inhibitors. This approach connects computational prioritization with mechanism-focused experimental validation.

Exploring the diversity and antimicrobial potential of lactic acid bacteria isolated from fermented black couscous (El-Hammoum), a traditional Algerian product

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Introduction

In many rural and Berber-speaking regions of Algeria, the "black couscous" known as El-Hammoum is a traditional product derived from fermented wheat, occupying a singular place in local dietary habits. Perceived as a remedy rather than a simple dish, it owes its reputation to its presumed nutritional and therapeutic properties, as well as its lengthy preparation process. Historically, wheat is stored in underground silos (Matmora), where humidity, water infiltration, temperature fluctuations, and anoxic conditions promote spontaneous fermentation that can last for several months or even years. This fermentation induces significant biochemical modifications and contributes to the development of a microflora dominated by lactic acid bacteria (LAB) and yeasts. The characterization of this microflora is essential for identifying strains of technological interest, particularly those capable of producing antimicrobial metabolites, especially bacteriocins. Bacteriocins are antimicrobial peptides synthesized by certain LAB, active against pathogenic or spoilage bacteria, and are of major interest for food biopreservation.

Objectives

This study aimed to isolate, identify, and characterize novel lactic acid bacteria strains from El-Hammoum, by evaluating their antimicrobial potential and their ability to produce bacteriocins.

Methods

Lactic acid bacteria were isolated using classical microbiological methods from three different El-Hammoum samples originating from three distinct Matmora. The isolates were characterized by Gram staining and catalase testing, and then identified by 16S rRNA gene sequencing. Their antimicrobial potential was assessed using the agar well diffusion method against indicator strains. Bacteriocin production was investigated by neutralizing the effect of organic acids and hydrogen peroxide, as well as by treating supernatants with proteolytic enzymes, in order to identify the peptide nature of the inhibitory agent and to confirm that it is indeed a bacteriocin-type compound.

Results

Molecular identification of the isolates revealed the presence of six bacterial genera: *Lactobacillus* (55%), *Lactococcus* (13%), *Pediococcus* (12%), *Enterococcus* (10%), *Leuconostoc* (6%), and *Streptococcus* (4%). Antimicrobial screening showed that several strains, particularly those belonging to the genera Lactobacillus and Enterococcus, exhibited significant inhibitory activity against the indicator strains tested. This activity was partially attributed to bacteriocin production, as confirmed by the persistence of inhibition after neutralization of acids and hydrogen peroxide, as well as by its sensitivity to proteolytic treatment.

Conclusions

These results suggest that El-Hammoum strains could represent a potential source of bacteriocins for food biopreservation applications. The observed microbial diversity confirms the interest of El-Hammoum as a source of lactic acid bacteria with technological and antimicrobial potential. These strains represent promising candidates for the development of starter cultures and biocontrol agents. Further studies are still needed to characterize the bacteriocins produced, identify their biosynthesis genes, and evaluate their stability and efficacy under real-use conditions, as well as their safety.

Keywords:

Lactic acid bacteria; El-Hammoum; Bacteriocins; Antimicrobial activity; Fermented wheat; Traditional Algerian food; Food biopreservation.

Antimicrobial peptides as signalling molecules in gut bacteria

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In the gut, effectors of the innate immune system such as antimicrobial peptides (AMPs) affect bacterial survival in a concentration-dependent manner. Given their therapeutic potential, most studies have focused on their bactericidal activity at lethal concentrations. *In vivo*, however, secreted AMPs do not necessarily reach inhibitory levels, as local concentration gradients naturally occur. The few studies performed at sub-inhibitory concentrations, mainly on pathogens, report modulation of bacterial signalling, including biofilm formation and virulence. The underlying cellular and molecular mechanisms remain largely unknown, despite their physiological relevance.

This project aims to determine whether AMPs function as signalling molecules and to characterise the responses they elicit in gut bacteria at physiologically relevant concentrations.

Quantitative proteomics of *E. coli* K-12 MG1655 exposed to sub-inhibitory concentrations of cationic AMPs of distinct origins (human LL-37 and the synthetic peptide C18G) revealed a substantial proteomic response. The response was strongly peptide-specific, and functional enrichment analysis identified modulation of pathways involved in central metabolism, biofilm formation, motility, chemotaxis and AMP resistance. Notably, approximately 60% of the regulated proteins are functionally uncharacterised, underscoring the need for mechanistic investigation.

Phenotypic assays confirmed biofilm formation and quorum sensing were differentially modulated by the two peptides. Screening of deletion mutants of selected candidate genes identified eight strains with altered growth in the presence of AMPs. Among them, a mutant lacking a conserved protein of unknown function was completely growth-inhibited by both LL-37 and C18G at otherwise sub-inhibitory concentrations. This mutant also displayed abnormally short cells, suggesting an impairment of cell division or separation. Together, these results support a signalling function for AMPs at sub-inhibitory concentrations, advance the understanding of bacterial response mechanisms, and identify a conserved protein of unknown function required for tolerance to LL-37 and C18G.

Functional Characterization and Antimicrobial Potential of Beneficial Bacterial Isolates for Microbial Biotechnology Applications

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The emergence of multidrug-resistant microorganisms has created an urgent need for the discovery of alternative antimicrobial resources and novel bioactive compounds. Beneficial bacteria constitute an important reservoir of functionally diverse microorganisms capable of producing metabolites and enzymes with antimicrobial potential. The present study aimed to characterize bacterial isolates based on their functional attributes and evaluate their potential as sources of antimicrobial compounds for biotechnology-oriented applications. A total of 10 bacterial isolates were subjected to comprehensive phenotypic, biochemical, and functional characterization. The isolates were screened for important microbial traits, including siderophore production, phosphate solubilization, ammonia production, hydrogen cyanide production, proteolytic activity, chitinolytic activity, and antagonistic potential. The screening revealed considerable functional diversity among the isolates, with individual strains exhibiting multiple beneficial traits. Siderophore production indicated the ability of selected isolates to efficiently sequester iron, whereas extracellular enzymatic activities suggested their capacity to interfere with competing microorganisms through hydrolytic mechanisms. Antagonistic assays further demonstrated differential inhibitory activity among the bacterial isolates, identifying a subset of promising candidates with enhanced antimicrobial potential. Molecular identification based on 16S rRNA gene sequencing assigned the isolates predominantly to members of the genera *Bacillus, Pseudomonas, Stenotrophomonas*, and related bacterial taxa. Notably, isolates affiliated with *Bacillus velezensis, Bacillus subtilis, Bacillus amyloliquefaciens*, and *Pseudomonas* spp. Displayed multiple functional characteristics associated with microbial antagonism. The coexistence of siderophore production, extracellular enzymatic activities, and antimicrobial effects suggests that these isolates may employ complementary mechanisms to suppress competing microorganisms. The identification of multifunctional bacterial candidates provides a valuable starting point for the characterization of their active antimicrobial constituents. Further biochemical, genomic, and proteomic investigations are warranted to determine whether peptide- or protein-associated antimicrobials, including bacteriocin-like compounds, contribute to the observed activity and to elucidate their underlying biosynthetic pathways. Overall, the study demonstrates the potential of environmentally derived bacteria as a source of bioactive antimicrobial compounds and provides a foundation for their further investigation in antimicrobial biotechnology and strategies addressing antimicrobial resistance.

Direct Detection of Cyclic Lipopeptides in Bacterial Culture via liquid-state NMR

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Introduction:

Antibiotic resistance is a growing global problem, highlighting the need for new antimicrobial compounds. Cyclic lipopeptides (CLiPs) are a promising class of natural products produced by bacteria from various genera, including *Pseudomonas*. Their discovery from bacterial cultures typically relies on liquid chromatography–mass spectrometry (LC-MS), which requires sample extraction and, in some cases, purification prior to analysis. Moreover, mass measurements alone are often insufficient to confirm that a candidate hit is a peptide, as other classes of natural products can exhibit similar masses.

Objectives:

To address these limitations, we aimed to develop an NMR-based screening approach for the direct detection of CLiPs in bacterial cultures.

Methods:

Bacterial cultures were grown using a ^{15}N-labeled nitrogen source, and 2D ^{1}H–^{15}N HSQC spectra were acquired directly from the cultures following the addition of deuterated solvent. The analysis targeted the amide (N–H) backbone region, providing a peptide-specific fingerprint without requiring extraction or purification.

Results:

As a proof of concept, the approach was applied to two orfamide-producing strains. Acquisition of interpretable N–H fingerprints required the addition of ACN-d_3, which improved the apparent solubility of the lipopeptides in the culture medium. The spectra obtained from the two strains showed consistently aligned peaks, demonstrating reproducible detection of the shared orfamide fingerprint across independent samples. These observations suggest that aggregation of CLiPs in the culture medium can substantially reduce their NMR detectability, while ACN-d_3 addition can improve signal visibility.

Conclusions:

Direct ^{1}H–^{15}N HSQC analysis of bacterial cultures provides a rapid, extraction-free approach for detecting CLiPs and generates a characteristic peptide backbone fingerprint that complements mass-based screening. The method may therefore help facilitate the early detection of CLiPs directly from bacterial cultures which can be applied in many fields.

The biogeography and antimicrobial potential of Scottish seagrass microbiomes

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Introduction

Seagrasses are key drivers of marine ecosystem health but are among the most threatened ecosystems globally. Seagrasses support diverse ecosystem services and host complex microbial communities that have been shown to have antimicrobial properties in situ. However, the composition, functional properties, and spatial variability of seagrass leaf microbiomes and the antimicrobial peptides they produce remain unstudied in the United Kingdom. Recent advances in metagenomic sequencing and artificial intelligence-based approaches now provide the opportunity to investigate the diversity of microbes and antimicrobial peptides across Scottish seagrasses.

Objectives

This project aims to investigate spatial variation in *Zostera noltii* (dwarf eelgrass) epiphytic microbiomes across Scotland, including microbial community composition and antimicrobial peptide diversity. In doing so, we will explore the biogeography of microbial antimicrobial peptides and how these vary across environmental conditions.

Methods

Seagrass epiphyte microbiomes were sampled from eight sites across Scotland in spring 2026, representing large variation in environmental conditions. Long-read metagenomic sequencing was used to generate metagenome assembled genomes for bacterial taxa and deep-learning based models were used to identify putative antimicrobialpeptides. Methods from phylogenetic community ecology were used to investigate how microbiome and antimicrobial peptide diversity varies across space and in relation to geophysical pressure from surrounding waters.

Results

We found substantial spatial heterogeneity in environmental conditions among Scottish seagrass habitats, reflecting variation in water quality and physical features of the catchments. Mirroring this variation, we found that microbial communities were spatially structured, which was associated with differences in the antimicrobial peptides produced.

Conclusions

This study provides insight into the ecological factors shaping Scottish seagrass microbial communities and their potential antimicrobial properties. This work contributes to defining the role of seagrass microbiomes in coastal ecosystem health and their potential as a source of novel antimicrobial compounds amidst the growing threat of climate change and antimicrobial resistance. More broadly, by combining environmental and metagenomic data, our study improves our understanding of how host-associated microbial communities vary across environmental gradients and how these structures AMP diversity and function. This will help streamline the identification and isolation of putative microbial sources of AMPs, while supporting the subsequent design and prioritisation of candidate peptides with improved predicted efficacy against target pathogens.

Investigating the IMD pathway in the bedbug *Cimex lectularius* and its function in the control of infections and mutualistic symbioses

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In insects, the innate immune system is based on two main responses: the humoral and cellular responses. The humoral immune response involves the production of antimicrobial peptides (AMPs) that protect insects against bacteria, fungi or viruses. In the case of infections by Gram-negative bacteria, AMP production is mainly mediated by the Immune Deficiency (IMD) signaling pathway. In *Drosophila melanogaster*, the IMD pathway is triggered upon recognition of bacterial peptidoglycan by the PGRP-LC receptor, which activates a downstream signaling cascade involving IMD and resulting in the activation of the transcription factor Relish, which in turn activates the expression of effector genes, including AMP-encoding genes.

Numerous insects rely on nutritional intracellular endosymbionts that are essential for their survival, growth and/or reproduction. We can thus wonder how their immune system can control symbiotic bacteria without eliminating them while defending against free-living pathogens. Nutritional endosymbiosis allows arthropods to thrive on unbalanced media such as the plant phloem, cereal grains or blood. For example, the bedbug Cimex lectularius, a blood-feeding human parasite, harbors the Gram-negative endosymbiont Wolbachia (wCle), which complements their diet in B vitamins, required for efficient reproduction and development.

Little is known about how the bedbug's immune system interacts with this obligate nutritional endosymbiont w*Cle*. Although early genomic studies reported the absence of several IMD components, suggesting that w*Cle* might be protected from the IMD pathway by its non-functionality, a recent work has identified elements previously thought to be missing, including the IMD protein itself (Nishide, Y. *et al.,* Sci Rep 2025). Therefore, we investigated whether the IMD pathway is functional following Gram-negative bacteria infection in *C. lectularius*, and whether it is involved in the control of the endosymbiont w*Cle*. To this end, we used RNA interference (RNAi) to silence *relish*, *pgrp-lc* and *imd* before challenging bedbugs with injection of free-living Gram-negative bacteria. The induction of a humoral immune response was assessed by RT-qPCR, following the expression of three AMP-encoding genes. The results show an extinction of the immune response when *relish, pgrp-lc* or *imd* is silenced, with a significantly reduced expression of the three studied AMPs, as compared to control conditions (*gfp* RNAi).

Next, we aimed to address whether the IMD pathway could also be involved in the control of the endosymbiont w*Cle*. Unexpectedly, this study led us to identify the presence of not one, but two *Wolbachia* strains in the bedbug population we were studying. w*Cle* was present with a prevalence of 100%, as expected and the other *Wolbachia massiliensis* was found with a much lower prevalence. In this context, *W. massiliensis* may either be more readily recognized by the host immune system or more sensitive to IMD-dependent effectors, such as AMPs, compared to *wCle*. In some cases of infection with the other *Wolbachia* strain, the absence of a functional IMD pathway may lead to an increase in the load of this strain, while *wCle* remains under control.

Finally, these results strongly support the existence of a functional IMD pathway in *C. lectularius* and demonstrate that this pathway plays a central role in the transcriptional activation of AMPs in response to gram-negative infection.

Ecological dynamics of the *Sepia officinalis* skin microbiome: from early life assembly to late-life *Vibrionaceae* bloom

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Introduction:

In aquatic organisms, particularly cephalopods, the skin and its mucus serve as crucial physical, chemical, and biological barriers against pathogens, directly reflecting the health status of the animal. This preliminary study investigates the dynamic development of the skin microbiome in the cuttlefish (*Sepia officinalis*) across its lifespan, with a specific focus on the proliferation and characterization of *Vibrio* populations associated with post-reproductive senescence and skin lesions.

Methods:

Skin and mucus samples were collected across multiple developmental stages: embryos, juveniles, one-year-old immature adults, mature adults during reproduction, and senescent individuals (healthy and ulcerated skin). Microbiome composition and succession were analyzed via 16S rRNA gene metabarcoding. To further characterize the *Vibrio* communities present during late-life stages, bacterial isolates were obtained using selective media, followed by full-genome sequencing using Oxford Nanopore long-read technology.

Results:

The cuttlefish skin microbiome undergoes progressive diversification over time, dominated primarily by Pseudomonadota and Bacteroidota, with phyla such as Patescibacteria and Mycoplasmatota appearing exclusively in mature adults. While early developmental stages exhibit low bacterial diversity, generic diversity doubles in immature adults and increases sixfold in mature adults. Post-reproductive senescence is marked by dysbiosis and skin ulcerations, coinciding with a significant surge in *Vibrio* species. Genomic analyses revealed a diverse array of Vibrionaceae, dominated by distinct strains of *Vibrio atlanticus*, alongside *V. harveyi*, *V. jasicida*, *V. scophthalmi*, and *Aliivibrio fischeri*.

Conclusion:

The skin microbiome of *S. officinalis* exhibits a tightly regulated succession linked to host age and physiological stage. The proliferation of diverse *Vibrionaceae* species during senescence, particularly within localized skin ulcers, suggests that opportunistic bacterial pathogens contribute significantly to the physiological degeneration observed prior to natural death in cuttlefish.

Synthesis of novel analogues of the natural alterin antimicrobial peptide as scaffolds for antibiotic renewal

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Antibiotic resistance is now a major threat to public health, as it reduces the effectiveness of the antibiotics currently available. In this context, it is essential to develop new therapeutic approaches, including antibiotics that can selectively act on resistant bacterial strains. Alterins (Fleury et al.) are macrocyclic lipopetides produced by marine bacteria of the genus *Pseudoalteromonas,* structurally related to polymyxins. While polymyxins display potent activity against Gram-negative bacteria, their clinical use is limited by nephrotoxicity and neurotoxicity.

This project is based on an innovative strategy aimed at inducing a selective reaction between antibiotics and the lipopolysaccharides of Gram-negative bacteria. To achieve this, the design and synthesis of alterin analogues are explored. The final aim is to build a library of alterin analogues in order to identify candidates with improved potency and a better safety profile.

The synthesis of unnatural amino acids (β-OH-Dab and Dhb), as well as their functionalization with hydrocarbon chains, either aromatic or aliphatic, was undertaken. One of the key structural elements of alterins is the presence of β-OH-Dab. Two multi-step strategies are currently being investigated to access this residue with orthogonal protecting groups: one involving a copper complex and the other using a boron complex. To validate a first strategy for the synthesis of the target peptides, a simplified analogue was synthesized in which Dab residues, Boc-Dab(Fmoc)-OH, were used to replace unusual β-OH-Dab. Another structural feature of alterins is a Dhb residue, obtained by dehydration of a threonine residue. Two hydrocarbon chains were then attached to these building blocks: a 10-carbon aliphatic chain, which showed the best biological effect in preliminary tests on natural alterins, and a biphenyl chain, previously reported as promising (Takiguchi *et al*.).

Finally, two linear analogues were prepared by solid-phase peptide synthesis (SPPS), both using an Fmoc/O*t*Bu strategy on a 2-chlorotrityl chloride resin, differing only in amino acid coupling times. The linear peptides were cyclized in solution using two coupling systems: EDCI/Oxyma with HATU, and PyBOP. This allows the isolation of 2 mg of each macrocyclic analogues with an LC-MS purity of 97%.

Obtaining two alterin analogues provides a proof of concept for the synthesis of these peptide macrocycles, opening the way to the construction of a wider analogue library. These first results will allow biological evaluation of the analogues to begin. The next step of the project will be to synthesize the β-OH-Dab residue and incorporate it into the macrocycle, ultimately enabling the design of selective antibiotics active on resistant Gram-negative strains

Genetic Code Expansion and Noncanonical Amino Acid Biosynthesis for Lasso Peptide Engineering

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Introduction

Lasso peptides are ribosomally synthesized and post-translationally modified peptides (RiPPs) whose threaded topology offers a scaffold for molecular engineering. Incorporation of noncanonical amino acids (ncAAs) can expand their chemical diversity [1,2]. Extending this approach requires access to the target building blocks and compatibility with both translation and post-translational maturation.

Objectives

To develop an approach combining ncAA biosynthesis, genetic code expansion and precursor peptide engineering for site-specific ncAA incorporation into lasso peptides, using microcin J25 (MccJ25) as a model system.

Methods

A diversified Methanomethylophilus alvus pyrrolysyl-tRNA synthetase (MaPylRS) library was subjected to positive and negative selection, followed by fluorescence-based screening with an amber-containing superfolder GFP (sfGFP) reporter [3]. Amber codons were introduced at selected positions in the MccJ25 precursor gene to investigate compatibility with peptide maturation. Complementary biosynthetic work aims to provide access to the selected ncAAs.

Results

Selection and screening identified candidate MaPylRS variants for further evaluation of ncAA-dependent amber suppression, substrate specificity and incorporation efficiency. Engineered MccJ25 precursor constructs were generated, and preliminary mass-spectrometric analyses detected candidate peptide products. Structural characterization is required to establish product identity, verify site-specific ncAA incorporation and confirm lasso topology.

Conclusions

This work provides an experimental basis for evaluating compatibility between engineered translation and lasso peptide maturation. Coupling these approaches with ncAA biosynthesis could broaden access to chemically diversified lasso peptides for subsequent investigations of peptide structure, physicochemical properties and antibacterial activity.

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Engineering of class I Lasso peptides as resistance blockers in human pathogens

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Introduction

Antimicrobial resistance is a major global health challenge, highlighting the need for novel antimicrobial strategies and targets. Lasso peptides are ribosomal synthesized and post-translationally modified peptides (RiPPs) characterized by a highly stable threaded topology and diverse biological activities. They are emerging scaffolds for new antibiotics development[1]. Emergence and spread of vancomycin resistance in Gram-positive human pathogens such as *Enterococcus* spp. and *Staphylococcus aureus* represent a significant therapeutic challenge. In this context, the class I lasso peptides, displaying two disulfide bonds, are of particular interest. Two such peptides, sviceucin and siamycin I, are able to attenuate virulence and restore vancomycin susceptibility in *Enterococcus* spp. by targeting the histidine kinases (HKs) FsrC and VanS of the respective two-component system (TCSs)[2]. While siamycin I exhibits good antibacterial activity and affects both vancomycin VanS type A and B resistance, sviceucin is a weak antibacterial and primarily targets VanS type B resistance. Previous engineering based on the natural diversity of class I lasso peptides identified a sviceucin variant V20I with broadened activity against VanS type A and enhanced VanS type B inhibition, while remaining non-antibacterial. This indicated the importance of the C-terminal residue of sviceucin in determining the HK specificity. Molecular docking further suggested that the V20I substitution of sviceucin engages stronger interactions with the VanS HK, via subtle conformational change[3].

Objective

This study investigates the impact of the C-terminus residue of sviceucin in suppressing vancomycin resistance in *Enterococcus*, with the aim to engineer class I lasso peptides with broadened and superior VanS HK inhibitory property.

Methods

A library of sviceucin variants was generated by substituting the C-terminus valine (V20) with all other proteinogenic amino acids, making use a seamless mutagenesis strategy that we developed previously[3]. Peptide production was achieved in the heterologous host *Streptomyces coelicolor*.

Results

Sixteen out of seventeen designed variants have been cloned and peptide production was confirmed for all of them by liquid chromatography coupled to mass spectrometry analysis. Two variants, V20A and V20H, have been successfully isolated to date, and were found to have lost the vancomycin resistance suppression activity, independent of the VanS types.

Conclusions and perspectives

This work underscores the high tolerance of C-terminus substitution by the sviceucin biosynthetic machinery, and implies a key role of this residue in the interaction with the VanS HK. Ongoing work includes optimization of culture conditions to increase production yields and subsequent peptide isolation for activity test. Available variants will be assayed against selected HKs controlling resistance to other antibiotics. These experimental data will be corroborated with computational analysis of peptide-HK interactions. Overall, this work will provide a blueprint to engineer class I lasso peptides as resistance blockers.

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In Silico Design and Characterization of Omiganan and R-Omiganan Constructs for Heterologous Expression in *Chlamydomonas reinhardtii*

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Omiganan, a synthetic cationic antimicrobial peptide of 12 amino acids derived from bovine neutrophils, exhibits potent activity against *Staphylococcus aureus* by disrupting cell membranes and inhibiting macromolecular synthesis. Combined with anti-inflammatory properties, these traits make it a promising candidate for atopic dermatitis. However, large-scale production of antimicrobial peptides remains challenging, highlighting the need for alternative recombinant expression strategies. Multimeric peptide constructs and heterologous expression platforms, such as *Chlamydomonas reinhardtii*, may represent alternative approaches for recombinant peptide production. This study aimed to characterize and structurally model omiganan and r-omiganan, exploring alternative three-copy constructs with and without linker sequences for potential heterologous expression in *C. reinhardtii*. Six peptide models were generated using AlphaFold 3, comprising the original sequences and their respective three-copy variants, with and without linkers. Physicochemical properties, including molecular weight, isoelectric point, net charge, and aqueous solubility, were assessed using ProtParam and PepCalc. Antimicrobial potential was evaluated using AMPA and AMP Scanner v2, while minimum inhibitory concentrations (MICs) were predicted using APEX against selected bacterial species. Additional antimicrobial activity predictions against *Staphylococcus aureus* ATCC 25923 were performed using DBAASP. Structural modeling suggested predominantly irregular conformations for monomeric omiganan and r-omiganan, whereas the three-copy constructs exhibited predicted α-helical regions and more pronounced folding. Linker incorporation further altered the spatial arrangement of the multimeric structures.

Physicochemical characterization revealed similar properties between corresponding omiganan and r-omiganan constructs. The monomeric peptides exhibited a molecular weight of 1.78 kDa, a net charge of +4, and good predicted aqueous solubility, whereas three-copy constructs showed increased molecular weight (5.30 kDa), net charge (+12), and reduced solubility. Linker-containing constructs exhibited a molecular weight of 6.25 kDa. APEX predicted lower MICs for the three-copy constructs without linkers, particularly r-omiganan-3 (21.39 and 27.34 µg/µL) and omiganan-3 (24.39 and 37.64 µg/µL) against *Escherichia coli* ATCC 11775 and *S. aureus* ATCC 12600, respectively. DBAASP predictions indicated antimicrobial potential for all six constructs, with higher scores for omiganan-3 (0.79) and r-omiganan-3 (0.76). All constructs received an AMP Scanner score of 1.0, while AMPA identified regions below the antimicrobial propensity threshold (0.225), primarily outside the linker sequences. Overall, the computational pipeline identified three-copy omiganan and r-omiganan constructs without linkers as promising candidates based on their predicted antimicrobial activity, despite their reduced aqueous solubility. Heterologous expression in *Chlamydomonas reinhardtii* and molecular dynamics simulations of peptide-membrane interactions are currently underway to further investigate their production feasibility and antimicrobial potential.

Candidalysin, a fungal cytolytic peptide, targets bacterial envelopes: effects on membrane integrity and surface properties in Gram-positive and Gram-negative bacteria.

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Candidalysin (CL) is a cytolytic peptide secreted by the hyphal form of *Candida albicans*,[1] a major opportunistic fungal pathogen. Its effects on host cells are well documented and include membrane permeabilization and activation of inflammatory pathways, ultimately leading to cell lysis.[2] In contrast, its interactions with bacteria remain poorly understood. To our knowledge, only one study,[3] has reported that CL can inhibit the growth of certain gut bacteria, suggesting that this fungal peptide may also shape bacterial communities. Given its positive charge, amphipathic structure and host membrane-disrupting properties, we hypothesized that CL interacts with bacterial envelopes and thereby contributes to the interplay between *C. albicans* and bacteria.

This study aimed to investigate the effects of CL on bacterial viability, membrane integrity and surface properties, and to elucidate the mechanisms underlying peptide–bacteria interactions. Two bacterial models with distinct cell envelope architectures were used: the Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli*.

We used a multi-scale approach combining microbiological, imaging and biophysical techniques. The potential effect of CL on bacterial growth was assessed by a minimum inhibitory concentration (MIC) assay, and cytoplasmic membrane integrity was evaluated by SYTO 9/propidium iodide staining. CL localization on both bacterial species was examined by immunofluorescence, and atomic force microscopy (AFM) was used to characterize bacterial surface topography. Finally, large unilamellar vesicles (LUVs) mimicking *S. aureus* and *E. coli* membranes lipid compositions were used to assess CL-membrane interactions through calcein leakage assays.

Because CL auto-aggregates in all tested culture media, no MIC could be determined. In phosphate buffered saline (PBS), where CL is soluble, it nonetheless induced visible bacterial aggregation, and as shown by SYTO 9/PI staining, CL induced membrane permeabilization in *S. aureus* (45% of cells) and *E. coli* (12%). At the cellular scale, confocal images showed that CL predominantly localized at the bacterial surface of both species, colocalizing with the membrane, with no clear evidence of internalization. Consistent with this, AFM imaging revealed CL-induced alterations after 6 hours of exposure of the bacterial surface topography, characterized by increased roughness (+6nm) and envelope irregularities. At the molecular level, CL triggered calcein leakage from LUVs mimicking the lipid composition of both *E. coli* and *S.aureus* membranes, indicating a direct interaction with bacterial membrane lipids and membrane permeabilization.

Interestingly, this leakage was reduced by the addition of PGN or anti-CL antibodies, suggesting that PGN, a bacterial cell wall component, can limit the availability of CL for membrane interactions, as anti-CL antibodies do.

These findings show that CL interacts with bacterial envelopes, inducing membrane permeabilization and surface alterations in both Gram-positive and Gram-negative bacteria, offering new insights into its potential contribution to *Candida*–bacteria interactions in polymicrobial communities. They also provide a molecular-scale view of the potential contribution of PGN to CL-membrane bacteria interaction, although further experiments are needed to confirm this role.

References :

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From development to animal applications: concatemeric bacteriocins AurA53x4 and EntPx4 against S. agalactiae and S. aureus

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Introduction

Class II bacteriocins are a diverse group of antimicrobial peptides with considerable potential as alternatives to conventional antibiotics. Their potent activity against major Gram-positive pathogens, including *Staphylococcus aureus* and *Streptococcus agalactiae,* combined with their amenability to genetic manipulation, makes them attractive scaffolds for antimicrobial engineering. Moreover, their potential application against pathogens affecting different animal production systems makes engineered bacteriocins particularly relevant within a One Health framework. Recombinant production of bacteriocins is often challenging due to their small size, being usually necessary to use fusion tags to increase their production and minimize proteolysis. Alternatively, bacteriocins can be produced as multidomain proteins, avoiding the use of any tag. Thus, in this study we developed a novel concatemerization-based strategy to generate bacteriocin-derived antimicrobials and explore their application in aquaculture and livestock.

Objectives

To develop a concatemerization-based strategy to engineer bacteriocin-derived antimicrobials for use against One Health-relevant pathogens *S. aureus* and *S. agalactiae*, both *in vitro* and *in vivo*.

Methods

Four concatemeric bacteriocins, PedPA1x4, SakPx4, AurA53x4 and EntPx4, were heterologously produced in Escherichia coli and initially evaluated against reference methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) and S. agalactiae strains. AurA53x4 and EntPx4 were selected for combination studies, subsequently including clinical MSSA and S. agalactiae isolates. Antimicrobial activity was assessed by minimum inhibitory concentration (MIC), fractional inhibitory concentration index (FICI), and BacTiter-Glo™-based bactericidal checkerboard assays. The selected combination was further evaluated in a *S. agalactiae* challenge model in Nile tilapia (*Oreochromis niloticus*) with or without heat stress, and in a field trial involving dairy cattle during the dry period by monitoring somatic cell count (SCC).

Results

All four concatemeric bacteriocins retained antimicrobial activity following recombinant production and purification. EntPx4 displayed MICs of 1.25 µM against all reference pathogens, whereas AurA53x4 inhibited S. agalactiae at 1.25 µM and MSSA and MRSA at 2.50 µM. Although FICI analysis indicated indifferent interactions between AurA53x4 and EntPx4, their combination showed a more favourable profile in bactericidal assays. High killing efficiencies were maintained across several combinations, with bactericidal activity reaching >95% at combined total concentrations of 0.31 µM for *S. agalactiae*, 0.63 µM for MSSA and 2.66 µM for MRSA in selected pathogen-specific conditions. A general dose-sparing effect was observed upon combination of EntPx4 and AurA53x4. In vivo, prophylactic administration of the AurA53x4-EntPx4 combination resulted in 75% survival following challenge with a clinical *S. agalactiae* isolate in tilapia, while antibiotic supplementation resulted in <5% of tilapia survival. In the ruminant field trial, bacteriocin treatment was associated with a 35.7% reduction in somatic cell count (SCC), compared to a 8.3% reduction observed upon antibiotic treatment.

Conclusions

This work demonstrates the potential of concatemerization as a versatile bacteriocin engineering strategy for the development of novel antimicrobial interventions based on class II bacteriocins for One Health solutions. The combination of AurA53x4 and EntPx4 generated promising antimicrobial activity in vitro and showed encouraging results in both aquaculture and livestock settings.

Identification of Antimicrobial Peptide (AMPs) Candidates as Potential Alternatives to Antibiotics against Major Bacterial Diseases in Mediterranean Aquaculture

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Introduction

In an era of ever-growing global human population, aquaculture is one of the key industries that plays a major role in supporting global food security. However, the occurrence of disease outbreaks, particularly those caused by bacterial pathogens, is one of the main limiting factors in the sector. These outbreaks not only contributed to significant economic losses but also increased the widespread use of antibiotics, which led to the emergence of antimicrobial resistance (AMR). Therefore, developing more sustainable alternatives to conventional antibiotics has become a pressing issue for the aquaculture industry. Given that antimicrobial peptides (AMPs) are well-documented to possess broad-spectrum antimicrobial properties and, unlike antibiotics, they exert the ability to modulate host immune responses, it is worth exploring their potential applications in aquaculture.

Objectives

The objective of this preliminary study is to screen eight recombinant AMPs, including four bacteriocins (EntPx4, Aur53x4, SakPx4, and PedPA1x4) and four host defense peptides (BD1x4, LAPx4, HBD3x4, and HD5x4), against four major bacterial pathogens in Mediterranean aquaculture: *Photobacterium damselae subsp. Piscicida, Vibrio harveyi, Vibrio anguillarum,* and *Lactococcus garvaie.*

Methods

Eight AMPs were recombinantly produced in *Escherichia coli* BL21(DE3) as a concatemeric polypeptide composed of four repetitions of each AMP and purified through IMAC. The antimicrobial performance of individual peptides was compared across bacterial species to identify promising candidates for further development. The evaluation of bactericidal activity of all pathogens following peptide treatment was carried out using the BacTiter-Glo™ Microbial Cell Viability assay by taking intracellular ATP levels as an indicator of metabolically active cells. This was performed against relevant aquaculture pathogens, namely *Photobacterium damselae subsp. Piscicida, Vibrio harveyi, Vibrio anguillarum,* and *Lactococcus garvaie.* Additionally, minimum inhibitory concentration (MIC) assays were done to determine the lowest peptide concentration to inhibit bacterial growth of *V. anguillarum* and *L. garvaie*.

Results

Although the bactericidal activity varied considerably among the tested pathogens and AMP candidates, HDPs' activity was shown to be dose-dependent, with greater activity at higher concentrations. Notably, the HDP LAPx4 and bacteriocin Aur53x4 consistently showed superior killing effects against all bacterial strains, whereas other peptide candidates displayed weaker and comparable activity. The efficacy of Aur53x4 was further supported by MIC results, where it performed the best among the bacteriocins when tested against *L. garvaie* (MIC = 1.25 µM). However, when tested against *V. anguillarum*, all bacteriocins were working equally effective (MIC = 1.25 µM). On the other hand, the MIC results of HDPs against *L. garvaie* showed that all peptides were equally effective at a concentration of 5 µM, whereas all HDPs showed lower MIC value (MIC = 1.25 µM) against *V. anguillarum.*

Conclusions

This preliminary study demonstrates the potential of selected antimicrobial peptides as agents to control bacterial diseases in Mediterranean aquaculture.