

ANTIMIC 2024



BOOK OF ABSTRACTS

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ORAL PRESENTATIONS

Innovative antimicrobial molecules of plant origin

Medicinal plants and food processing by-products: An alternative source for antimicrobials

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Abstract :

The interest in natural antimicrobials obtained from different sources including bacteria, plants, animals, fungi, etc., which are alternatives to the use of synthetic chemicals, is increasing day by day, due to the consumer's demand for environmentally friendly production systems and products with clean labels. Natural antimicrobials cover a wide variety of compounds including phenolics, terpenes, bacteriocins, peptides, enzymes/proteins, natural polymers, fatty acids, organic acids and mixtures of bioactive compounds such as essential oils and plant extracts. Food processing by-products and medicinal plants are considered as natural antimicrobials with their bioactive compounds used to preserve food from spoilage and chemical oxidization processes.

This conference aims to summarize recent published results, focusing on the potential of medicinal plant and food processing by-products extracts or their bioactive compounds as natural antimicrobial. In this context, our recent study Khelouf et al. (2023a) reported that carob pulp extract has a high concentration of phenolic compounds (isorhamnetin and quercetin) with significant antioxidant activity, antimicrobial property against *Staphylococcus aureus* and *Bacillus subtilis*, and antifungal effect against *Aspergillus Niger* and *Candida albicans*. Khelouf et al. (2023b) mentioned that olive leaves extracts showed excellent antifungal activity against *Aspergillus Niger* ATCC16404. In the food field, lycopene extracted from tomato pomace, showing good results in preserving freshness and extending the shelf-life of the chicken meat (Jabri Karoui et al., 2023b).

Several research have been conducted to find out the antimicrobial potential of bioactive compounds from plant and food processing by-products sources. In conclusion, natural antimicrobials can pave the way for more effective, safe, and sustainable food preservation strategies in the future.

Keywords : Medicinal plants, food processing by-products, bioactive compounds, natural antimicrobials, food preservation, phenolics, essential oils.

Natural products to fight coronavirus infections

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Abstract :

Coronaviruses (HCoV) can cause mild to severe respiratory illness in humans. Despite significant advances in the understanding of how these viruses work and the creation of effective vaccines, these viral diseases remain a public health concern due to the risk of recurring epidemics, particularly linked to the emergence of variants. The COVID-19 pandemic, caused by SARS-CoV-2, has highlighted the lack of specific antivirals available against HCoVs and the need for better clinical management of these infections. Our overall strategy is to identify pan-coronavirus antivirals, taking advantage of natural structural diversity. In our studies, different types of specialized metabolites have shown their antiviral potential against coronaviruses, including phenolic compounds, terpenoids and alkaloids. This presentation will be an opportunity to present some collaborative work carried out which has highlighted the anti-coronavirus potential of certain substances of natural origin and synthetic analogues. Acknowledgements: Authors gratefully thank the different funding organizations for PhD theses of Malak Al Ibrahim, Asma Hakem, Abda Ba and Imelda Raczkiwicz, as well as for research programs ANR Flash FlavoCoV, CNRS prematuration and PHC Utique, in particular Hauts-de-France Region, University of Lille (I-Site), Campus France and CNRS Innovation.

References: [1] Hakem, A, Desmarets, L, Sahli, R, Ben Malek, R, Camuzet, C, François, N, Lefèvre, G, Samaillie, J, Moureu, S, Sahpaz, S, Belouzard, S, Ksouri, R, Séron K, Rivière C. Luteolin isolated from *Juncus acutus* L., a potential remedy for human coronavirus 229E. *Molecules* 2023, 28, 4263. <https://doi.org/10.3390/molecules28114263> [2] Al Ibrahim, M, Akissi, ZLE, Desmarets, L, Lefèvre, G, Samaillie, J, Raczkiwicz, I, Sahpaz, S, Dubuisson, J, Belouzard, S, Rivière, C, Séron, K. Discovery of anti-coronavirus cinnamoyl triterpenoids isolated from *Hippophae rhamnoides* during a screening of halophytes from the North Sea and Channel coasts in northern France. *Int. J. Mol. Sci.* 2023, 24, 16617. <https://doi.org/10.3390/ijms242316617>

Keywords : Anti-coronavirus agents; SARS-CoV-2; Plant; Natural Products; Synthetized derivatives.

Innovative antimicrobial molecules of animal & microbial origin

Fish peptides as templates for the design of novel antimicrobials

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Abstract :

Food safety is a public health priority. Control over food microflora is essential but challenging owing to the upsurge of microbial resistance. In search of solutions, health specialists and food industries are looking for new and safer types of antimicrobials from natural sources. Numerous marine-derived compounds have been proven to harbor antimicrobial properties. Previous works revealed the potential of fishery wastes' by-products, as a promising source of antimicrobial peptides (AMPs) whilst their mode of action has not been entirely determined yet. The identification of the structure and bio-functionality of novel sources of fish peptides lead to the elucidation of their activity to optimize their use in food and enhance their quality. The main goal of our research was to study recently discovered fish AMPs by our team and elucidate their structure activity relationship. Specific objectives were conducted to determine the relationship between the primary structure or spatial conformation and antimicrobial activity of fish AMPs, and analogues thereof and to study the fish AMPs-microbial membrane interaction. The native fish AMP and different analogs were designed and synthesized to study the impact of net charge, hydrophobicity, amphipathicity and secondary structure on both antibacterial and antifungal activities. Experiments with the native fish AMP and its synthetic analogs revealed that their antimicrobial action was intimately related to their cationicity and hydrophobicity and that although they induce membrane permeabilization in the microorganisms tested, their mechanism of action appears to be at least partially related to intracellular targets. The results contribute to innovative developments toward the design of novel antimicrobials using marine peptides as templates, by an integrated systems approach to structure-function relationships, providing the baseline and tools to develop new antimicrobial agents to maintain the ability to fight the threat of microbial infection.

Keywords : Fish; Antimicrobials; Peptides; Structure-activity.

Turkey cruor as a source of bioactive peptides: How pH and peptic hydrolysis duration could affect the peptide population?

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Abstract :

The consumption of meat protein will be increasing by 14% and the poultry meat will constitute 41% of all the protein from meat sources in 2030 [1]. Consequently, global pressure is growing to minimize the environmental impact of slaughterhouses. One promising way to address this issue is to valorize by-products such as blood, by the production of bioactive peptides using enzymatic hydrolysis [2]. To the best of our knowledge, no research has been previously performed regarding the peptic hydrolysis of turkey cruor which contains, after blood centrifugation, hemoglobin. This project presents the impact of pH (2, 3, 4, and 5) and enzymatic hydrolysis duration (T30 min and T180min) of turkey cruor on the peptide population and their bioactivity (antimicrobial and antioxidant). Four bacterial strains *Listeria ivanovi* (HP B28), *Salmonella enterica ssp Newport* (ATCC 6962), *Campylobacter Coli* (ATCC 33559) and an *Escherichia coli* (MP 4100), two filamentous fungus strain, *Mucor racemosus* (LMA-722) and *Paecilomyces spp* (5332-9a), a yeast strain *Rhodotorula mucilaginosa* (27,173) were tested to evaluate the antimicrobial activities of hydrolysates at two different durations of hydrolysis (T30 min and T180min). Orac test was performed to evaluate the antioxidant activity. For the results, antifungal and antiyeast activity was observed: the lowest Minimum inhibitory concentration (MIC) for *Paecilomyces spp* was observed at pH2 T30min and pH3 T30min (0.63 mg/ml), for *Mucor racemosus* and *Rhodotorula mucilaginosa* the lowest MIC was observed at pH3 T30min (1.25 mg/ml and 0.63 mg/ml respectively). Furthermore, no antibacterial activity was detected against the four bacteria tested. For the antioxidant activity results, it was found that the concentration (0.313 mg/ml) of hydrolysates produced at pH 3 T30 min presents the highest antioxidant activity (199.67± 0.49 µM Trolox Eq). Based on these results, one can speculate that pH3 T30 min presents the most promising condition to produce antifungal, antiyeast and antioxidant peptides.

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Keywords : Turkey cruor; Hemoglobin; Enzymatic hydrolysis; Antimicrobial and antioxidants peptide.

Characterization of chicken cruor-derived hydrolysates produced through enzymatic hydrolysis and evaluation of the feasibility of their use as natural antimicrobials

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Abstract :

Chicken blood is a rich protein source that can be upgraded to generate value-added products. Each year, 48 million liters of blood are produced during chicken slaughter in Canada. About 40% of the total volume of blood is composed of cruor, which mainly contains hemoglobin. Upon hydrolysis of cruor, bioactive peptides with antimicrobial and antioxidant activity may be obtained and can be used as an alternative to chemical preservatives. After collecting chicken blood from slaughterhouses and separating the cruor by centrifugation, 180 minutes of peptic hydrolysis were performed at pH 2, 3, 4, and 5. Then, peptide population, sequence, enzyme mechanism, and degree of hydrolysis (DH%) were determined to evaluate the hydrolysates generated during the process. The hydrolysates were then tested for their antifungal and antibacterial activity against three major pathogenic bacteria of poultry meat (*Listeria ivanovii* HP B28, *Campylobacter Coli* ATCC 33559, *Salmonella enterica ssp Newport* ATCC 6962), as well as one yeast (*Rhodotorula mucilaginosa* 27,173) and two molds (*Paecilomyces spp.* LMA-722 and *Mucor racemosus* LMA-722). Next, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) of hydrolysates were determined. The highest DH% was recorded at pH 2, which was not significantly different from pH 3 ($p < 0.05$). Inhibitory effects were observed for hydrolysates produced at pH 2 and 3 after 30 and 180 minutes of hydrolysis against *Paecilomyces*, *Rhodotorula mucilaginosa* and *M. racemosus*. Although all hydrolysates produced showed no antibacterial activity, some new potential antimicrobial peptides, according to bioinformatics tools, were identified which can show antibacterial activity after separation and purification. This project aimed to valorize blood, a by-product from slaughterhouses, into high-added value peptide fractions with effective antimicrobial properties in the context of a circular economy. The study examined the possibility of creating a natural preservative, in addition to reducing waste.

Keywords : Chicken cruor; Antifungal; Antimicrobial; Enzymatic hydrolysis; Bioactive peptide.

Bacterial lipopeptides as promising antimicrobials: Structure-activity studies for pharmacological optimization

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Abstract :

The increase and spread of antibiotic resistance have become a major challenge in the prevention and treatment of bacterial infections. Faced with this critical public health problem, the development of innovative antimicrobials has become a global priority. Among the most promising alternatives to antibiotics, our team is particularly interested in antimicrobial peptides produced by bacteria such as lipopeptides. Lipopeptides demonstrate very interesting properties such as high activity, diversified spectrum of action, and original modes of action that are different from clinically used antibiotics. Despite their enormous potential in the food, animal husbandry, veterinary, and pharmaceutical sectors, the use of these lipopeptides is often limited by the difficulties associated with their production and/or stability. To overcome these limitations, our team uses a combination of synthetic and peptidomimetic approaches to produce these peptides but also to carry out structure-activity studies allowing the development of analogs with improved stability and pharmacological properties. This strategy was previously applied to different bacteriocins and allowed us to develop efficient synthetic routes for large-scale production and generate analogs with improved properties. Building on this success, the strategy was recently applied to some promising lipopeptides and the results showed that we were able to produce analogs with attractive properties such as high selectivity against Gram-positive or Gram-negative bacteria, antibiofilm activity, resistance to proteases, and reduced cytotoxicity. The study also showed that some synthetic lipopeptide analogs exhibited activity against multidrug resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). Our work on the total synthesis and structure-activity studies of the lipopeptides humimycin and glycinocin will be presented and discussed in the light of the results obtained.

Keywords : Antimicrobial peptides; Lipopeptides; Peptide synthesis; Structure-activity relationship study; Antimicrobial resistance.

Marine Bacteria-Derived Pigments: A Promising Approach towards Biofilm Control and Beyond

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Abstract :

Antimicrobial agents, like antibiotics, play a crucial role in treating infections caused by pathogenic bacteria. However, the widespread development of bacterial resistance poses a growing threat, and only a few new molecules have been developed to combat this issue. By 2050, projections suggest that infections could become the leading cause of death worldwide, highlighting the urgent need to discover effective compounds as alternatives to conventional antibiotic therapies, particularly those targeting biofilms. Fortunately, marine environments hold immense potential with their wealth of unexplored bioactive molecules, including halogenated furanones, pigments, and antimicrobial peptides. The project delves into the exploration of antibiofilm properties of natural pigments derived from specific marine bacteria and/or archaea isolated from the Atlantic coast (La Rochelle, France). These characterized bacterial pigments will serve as the foundation for the development of bioinspired technologies for treating human diseases caused by biofilms. This multifaceted project encompasses three key steps. First, the creation of a natural pigment library: Marine bacteria have already been collected, identified, and are currently being used to establish a comprehensive library of natural pigments. In a second way, the production and the evaluation of pigments will be evaluated. Eco-responsible extraction and purification methods will be employed to produce the pigments. Subsequently, their phototoxic and antibiofilm activity will be thoroughly evaluated. And then, we have plan to valorised these new molecules by testing the pigments against bacterial biofilm models. Additionally, the project will explore the anticancer activity of the pigments using cancer cell lines and evaluate their anti-inflammatory effects on macrophage models. In conclusion, the project embodies an innovative and promising approach to addressing major public health issues. We anticipate the discovery of novel marine-derived bioactive molecules, the development of new therapies to combat multidrug-resistant bacterial infections and cancer, and the effective utilization of marine resources from Charente-Maritime.

Keywords : Antimicrobial; Biofilm; Marine-derived; Pigments; Antibiotic resistance.

Cultivating microbial dialogues with high-throughput precision: mapping dynamic ecological interactions in the dairy microbiome

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Abstract :

To engineer efficient microbial management strategies in the food industry, knowledge of microbial interactions is crucial. In fact, microorganisms live in communities where they influence each other in several ways, including their response to stress. To date, research on microbial interactions has focused primarily on the production of antagonistic metabolites. Although this aspect is interesting, it does not account for the complexity of bidirectional microbial interactions and their dynamics. This work explores the interactions between a collection of 24 reference strains of interest from the Lactic Acid Bacteria Network (RBL) and 70 microorganisms isolated from spoiled dairy products. Using a colony-handling robot and image analysis, bidirectional microbial interactions were measured at high throughput on solid media containing skim milk. A total of 1142 binary interactions were mapped and classified according to their ecological type at 12 h intervals over a period of 7 days. Our results show that the interaction types are dynamic. In addition, the RBL strains used often promoted the growth of dairy isolates, including isolates belonging to the genera *Acinetobacter*, *Klebsiella*, *Proteus* and *Providencia*. In contrast, the RBL strains tended to negatively impact each other. Furthermore, cooperating RBL strains tend to have more similar ecological behaviours toward dairy isolates than competing strains, pointing toward the existence of microbial cliques. These results constitute an unprecedented dataset that contributes to our understanding of microbial interactions at the system level. They open up new opportunities for the application of these strains in the dairy sector and for the formation of bioprotective consortiums. Future research will focus on the validation of these interactions in dairy products.

Keywords : High-throughput culturing; Microbial interactions; Lactic acid bacteria; Microbial contaminants.

New insights on how bacteriocins can modulate colonic microbial ecosystems: a case study of avian colonic microbiota

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Abstract :

In this study, we used a continuous in vitro fermentation model, PolyFerm S, to reproduce the chicken cecum microbiota and investigate the impact of three bacteriocins, namely microcin J25, nisin Z, and pediocin PA1, along with the antibiotic bacitracin, on the ecology and metabolism of the chicken cecal microbiota. The stability of the bacteriocins in this complex matrix was evaluated using microtitration and agar diffusion assays over 48 hours. It was shown that microcin J25 displayed potent inhibition activity against *Salmonella enterica* Newport, remaining active even after 24 hours of continuous fermentation. In contrast, the inhibitory activity of pediocin PA1 ceased after 8 hours, while no inhibition was observed with nisin Z, using *Listeria ivanovii* as a target. These results were confirmed by LC-MS/MS analysis, which allowed the detection of the different bacteriocins, as well as their degradation forms. Analysis of the most relevant short-chain fatty acids (scfa) revealed a significant decrease of butyric acid 24 hours after the initial inoculation of nisin Z. Propionic acid has significantly decreased 48 hours after the inoculation of bacitracin. Analysis of 16S rRNA gene metabarcoding is underway and should provide relevant information regarding the impact of the different bacteriocins on microbiota composition and modulation of scfa-producing strains.

Keywords : Antimicrobial; Bacteriocins; Gut Microbiota; Chicken; Omics.

Bioprospecting strategy for the discovery of antimicrobial compounds from a lichen microflora

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Abstract :

Antimicrobial Resistance (AMR), one of the main concerns of the World Health Organisation [6] occurs in human, animal or plant pathogens, and is one of the main targets of The One Health approach [2]. One of the strategies to combat AMR is therefore to search for antimicrobial compounds in under-examined ecological niches. The lichen symbiosis, as an external structure, forms a micro-ecosystem for microorganisms that participate in the holobiont chemical defence as a third symbiosis partner. Our research focuses on the microflora of several samples of *Rhizocarpon geographicum* to discover active compounds against two phyto-pathogens: *Erwinia amylovora* [7] and *Venturia inaequalis* [3], and two human pathogens (*Pseudomonas aeruginosa*, *Escherichia coli*). These samples have been harvested in coastal and terrestrial environments, subjected to different biotic and abiotic stresses. These environments have led to the development of 500 original bacterial strains [5] that can produce a variety of potentially active chemical structures [1,4]. Our process combines i/ the culture of 286 lichen-associated bacteria on a suitable medium, ii/ harvesting of the supernatant and pellet from each culture at an appropriate phase for the production of specialized metabolites, and iii/ evaluation of the inhibition potential of these extracts. Initial results performed on 150 freeze-dried supernatants highlighted the inhibitory activities of 17 supernatants against the targeted pathogens. The results of bioguided fractionation of the four most active strains against the targeted pathogens to isolate antimicrobial compounds will be discussed.

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Keywords : AMR; Lichen microflora; Bioguided fractionation; Phyto-pathogens.

Combatting *Clostridium* in the milk sector with bacteriocins: what if it starts from silage?

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Abstract :

Clostridium spp. are gram-positive, mostly obligate anaerobic, sporulating butyrate bacteria that flourish in low-sugar and soil-contaminated silage. *Clostridium* spores in silage are a major concern for dairy farmers since their spores can survive milk and cheese processing, leading to significant dairy product defects. Given the importance of silage in livestock and agricultural production systems, new strategies are required to preserve the silage production from butyric spoilage. The purpose of this study is to investigate the antimicrobial activity of different bacteriocins against *Clostridium* species isolated from silage. A total of 30 *Clostridium* species were isolated and identified by 16S rRNA sequencing. They were characterized in terms of their metabolic activity, capacity to produce gas and organic acids, resistance to antibiotics and role in cheese and silage spoilage. Their sensitivity to three potent natural antimicrobials namely nisin, enterocin and brevibacillin were studied by determining the minimum inhibitory concentrations (MIC), using the agar diffusion and microtitration tests. We have shown that most of the *Clostridium* species were gas and butyrate producers and cause significant late-blowing defect. All isolates are susceptible to tested antibiotics and were inhibited at different extend to the three bacteriocins tested with MIC values ranging from 12.5 to less than 0.19 µg/ml. This work also aims to produce a silage that has significant presence of *Clostridium* to simulate a real-life butyric silage. The butyric silage was made providing a propitious environment for the growth of *Clostridium*s to make sure the further studies on silage is well-considered. Overall, this study provides solid scientific evidence about the potential of bacteriocins as an effective solution to control *Clostridium* in the milk sector.

Keywords : Bacteriocin; Silage; *Clostridium*; Microbiota; Dairy Products.

Antimicrobial activity of nasal *Staphylococcus* isolates from wild rabbits and potential effect on nasal microbiota

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Abstract :

Background : *Staphylococcus* is a common commensal bacteria of humans and animals and can produce bacteriocins.

The objective was to detect antimicrobial activity (AA) in coagulase-positive and coagulase-negative staphylococci (CoPS and CoNS, respectively) from wild rabbits and to characterize them.

Methodology : A collection of 93 SCoN and 12 SCoP recovered from nasal samples of 54 wild rabbits were tested for AA against 15 indicator bacteria by Spot-on-lawn method, to detect bacteriocin-producing-staphylococci (BP-S). In addition, the AA of BP-S was analyzed against their cohabitant isolates (intra-sample) and 28 Gram-positive and -negative representative bacteria of the nasal microbiota of the 54 studied rabbits (inter-sample). Moreover, cross-immunity assay was performed among BP-S. Whole-Genome-Sequencing (WGS) analysis was performed (Nanopore-technology) on three BP-S (*S. pseudintermedius* X8119 and X8135; *S. xylosus* X8178). Bacteriocin gene clusters was predicted (antiSMASH/BAGEL4) and complete genetic characterization of these isolates was performed.

Results : Thirteen of 105 staphylococci tested (12%; 7 CoNS and 6 CoPS: *S. pseudintermedius*) exhibited AA against any of the 15 tested indicators. Eight BP-S showed AA against representative strains of the nasal rabbit microbiota. Ten BP-S showed AA against other BP-S. By WGS, bacteriocin gene clusters were detected in *S. pseudintermedius* X8119 (SubtilosinA, firstly described in *Bacillus subtilis* and a class III lantibiotic) and *S. pseudintermedius* X8135 (operon for Bottromycin, bacteriocin commonly found in *Streptomyces* sp.). The two *S. pseudintermedius* isolates belonged to new sequence-types. Regarding resistome, blaZ and mph(C) genes were identified in two BP-S. Additionally, the icaC virulence gene was found in strain-X8135.

Conclusion : The nasal staphylococci of wild rabbits, both CoNS and CoPS, produce substances with AA that may play a role in modulating their nasal microbiota. Moreover, the presence of bacteriocins typically found in other soil-related genera reveals a potential transmission between soil and rabbit nasal microbiota and their genomic content.

Keywords : *Staphylococcus*; Bacteriocins; Microbiota; Modulation.

Fungal Warriors: looking for antifungal microorganisms to combat fungal contamination in maple syrup production and beyond

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Abstract :

Fungal contamination poses a significant challenge in maple sap collection tubes and postproduction processes, affecting the quality and shelf life of maple-derived products. As an alternative to chemicals, the use of antifungal microorganisms emerges as a promising strategy to combat these challenges. This study aims to identify microorganisms with antifungal properties and to elucidate their mechanisms of action. Using a high-throughput method employing a robotic platform, we screened 1,022 microorganisms isolated from maple sap. These microorganisms were tested individually and in 3,198 pairwise combinations against a diverse panel of food spoilage yeast. While co-culturing microorganisms rarely enhanced antifungal activity, interaction-mediated suppression was more prevalent in our dataset. However, this screening process identified strains with distinct antifungal properties. Subsequently, we conducted a comprehensive genomic analysis of nine *Pseudomonas* strains exhibiting antifungal activity. By examining their genetic makeup, we uncovered key genomic features associated with their antifungal capabilities. Additionally, our genomic studies led to the discovery of three new species of *Pseudomonas*, including one that possess a characteristic phenazine-producing biosynthetic gene cluster. Presently, we are employing a functional genomics approach to further elucidate the mechanism of action of these antifungal strains. Ultimately, our goal is to combine strains with complementary modes of action, forming a consortium to reduce the likelihood of developing fungal resistance. Therefore, this research contributes to the identification of promising candidates for biocontrol or biopreservation applications against fungal contamination in acericulture and other food environments.

Keywords : Antifungal; *Pseudomonas*; New species; Whole genome sequencing; Maple sap.

POSTERS

Innovative antimicrobial molecules of plant origin

Exploring the bioactive effects and functional properties of peptides derived from brewer's spent grain proteins.

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Abstract :

To reduce pressure on climate change, using fewer fossil fuels and other natural resources, valorization of agri-food by-products in a circular economy context represents one of the most promising directions. Moreover, recent awareness of the connection between global population growth, health issues, and climate change, as well as food provision, has led to an increase in interest in alternative protein sources. Brewers' spent grain (BSG) is a source of plant protein, representing about 85% of all by-products generated in the brewing sector, accounting for 18–30% of proteins. Therefore, it is very interesting to explore their functional and bioactive potential by hydrolyzing these proteins to generate new and more promising means of valorization. Thus, this study proposes to produce a protein hydrolysate obtained from BSG protein concentrate and assess its functional and bioactive properties. The BSG protein concentrate was hydrolyzed with trypsin and chymotrypsin for 5h with a 3% protein solution. The degree of hydrolysis was then assessed by the O-phthalaldehyde (OPA) method, and RP-HPLC-MS/MS were used to identify the peptides produced. After hydrolysis, functional properties, including solubility, emulsifying capacity, foaming capacity, viscosity, color, and water retention power of the hydrolyzates at different hydrolysis stages were explored. Bioactive properties including antioxidant activity, antimicrobial potential and potential health benefits such as anti-diabetes and anti-hypertensive activity have also been evaluated. Additionally, the cytotoxicity tests were carried out on the obtained hydrolysates. These peptides can therefore have a high application potential as an ingredient in developing functional foods and nutraceuticals.

Keywords : Brewers spent grains; Proteins; Functional properties; Bioactive peptides.

Innovative antimicrobial molecules of animal origin

Influence of electric current conditions on peptide migration and fraction antimicrobial activities during electro dialysis with ultrafiltration membrane: a comprehensive machine learning-peptidomic study

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Abstract :

Enzymatic hydrolysis of blood, by generating antimicrobial peptides, is a promising avenue to produce high added-value new products, in a circular economy context. However, activities of such complex solutions can be limited by the highly diverse peptide population. Electrodialysis with ultrafiltration membrane (EDUF), separating peptides based on their charge and molecular mass, is an eco-efficient strategy to obtain fractions with enhanced bioactivities and increased purity. While continuous electrical current (CC) is conventionally applied, alternative modes like pulsed electric field (PEF) and polarity reversal (PR) can modify mineral selectivity, mitigate fouling, and optimize energy consumption. To date, no studies investigated effects of different electrical current conditions on peptide migration. In this context, five conditions were explored - CC, PEF ratio 1 and 10, and PR ratio 1 and 10 - on a well-characterized discolored porcine cruor hydrolysate. Using UPLC-MS/MS, migration rates were calculated for 150 peptides and 45 peptide physicochemical characteristics were extracted using bioinformatic tools. Employing a machine learning approach, a regression tree model was applied for each electrical conditions to establish links between peptide migration rates, physicochemical characteristics, and specific electric current conditions. Results revealed distinct root nodes, with molecular mass identified as the major feature for PR 1, and pI for CC, PEF 10, and PR 10. PEF 1 exhibited an intermediate regression tree. Tree comparisons helped evaluate similarities among electrical current conditions and predict key physicochemical characteristics influencing the selective migration of peptides. This study proposes a tentative explanation of the phenomena involved, such as effects on the diffusion boundary layer and concentration polarisation. Antimicrobial activities were assessed post-separation and revealed how the current conditions, by modifying the peptide migration, modulated the antimicrobial activities towards antifungal or antibacterial. Moreover, under specific electrical conditions, EDUF showed the capacity to produce two valuable fractions simultaneously with different antimicrobial activities.

Keywords : Antimicrobial; Peptides; Separation; Co-product.

Unveiling potential antimicrobial peptides in dairy wastewater: enzymatic hydrolysis of milk proteins for the generation of bioactive peptides

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Abstract :

The dairy industry generates substantial volumes of wastewater, comprising primarily white and cleaning wastewater derived from rinsing and cleaning-in-place procedures. The current study focuses on the hydrolysis of milk proteins from white wastewater, as an opportunity to valorize this dairy waste. Four different enzymes were tested (pepsin, trypsin, pronase E, and thermolysin) and the hydrolysis performances were analyzed. The degree of hydrolysis values, ranging from 2% to 13%, influenced the protein/peptide content in each hydrolysate. UPLC-MS/MS characterization identified differences in peptide sequences among hydrolysates produced by the four enzymes. The effect of hydrolysis duration was also explored with a comparison between these peptide populations with notable differences observed between 30 and 240 minutes of hydrolysis. Notably, 555 peptides were identified after 30 minutes, increasing to 693 after 240 minutes. After 240 minutes of hydrolysis, the number of peptide sequences identified was different according to the enzyme used: 181 peptides from thermolysin hydrolysis, 153 from pepsin, 126 from trypsin and 83 from pronase E. The main characteristics of these peptides were analyzed revealing 17 known antibacterial peptides prominently identified after 240 minutes of both pronase E and thermolysin hydrolyses. Despite their identification, these peptides were deemed insufficient in concentration to inhibit tested strains in antimicrobial assays, including *Clostridium tyrobutyricum* and *Pseudomonas aeruginosa*. Furthermore, peptides derived from Pronase E 30min, Pronase E 240 min, and Thermolysine 240 min. exhibited notable antifungal activity against *Mucor racemosus* at an MIC of 2.5 mg/ml of proteins, while no activity was observed against *Penicillium commune*. In future directions, electro dialysis with ultrafiltration membrane (EDUF) emerge as a prospective avenue to concentrate the potential antimicrobial peptides produced after 4 hours of hydrolysis with pronase E and thermolysin. Additionally, a parallel protocol is currently underway, focusing on the assessment of diverse bioactivities, including antioxidant and antihypertensive bioactivity, within the fractions generated through the four enzymatic conditions.

Keywords : Enzymatic hydrolysis; White wastewater; Antimicrobial peptides; Circular economy.

Innovative antimicrobial molecules of microbial origin

M1

Characterization of a methyltransferase for catalyzing iterative N-methylations at the leucinostatin termini in *Purpureocillium lilacinum*

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Abstract :

Leucinostatins are a family of nonapeptide antibiotics, derived from the well-known biocontrol fungus *Purpureocillium lilacinum*. They exhibit a diverse spectrum of biological activities that target a variety of pathogens, cancer cells, and protozoans. To enhance their biological efficacy, our research focuses on enhancing their bioactivity through structural modifications. Our initial investigations focused on the biosynthetic gene cluster of leucinostatins, an N-methyltransferase (NMT), LcsG, aroused our interest due to the extensively reported effect of methylation on molecular bioactivity. Through gene deletion, purification of recombinant protein, and in vitro assays, LcsG is revealed to be responsible for forming NH₂, NHCH₃, and N(CH₃)₂ groups to the C-terminal of leucinostatins. This work also led to the identification of novel methylated leucinostatins that exhibit better bioactivities compared to their parent molecules. Further research into the enzymatic mechanism of LcsG employed multiple sequence alignments and site-directed mutagenesis, revealing that LcsG shared a highly conserved S-adenosylmethionine (SAM) binding pocket. These analyses also identified two potential active residues, D368 and D395. Additionally, validation through diffdock and molecular dynamic simulations corroborated these observations, demonstrating that the targeted N could be docked between D368 and D395. It is noteworthy that NMTs are commonly reported as a domain within nonribosomal peptide synthetases (NRPSs), where they typically catalyze the mono-methylation of side chains or peptide bonds in nonribosomal peptides (NRPs). However, LcsG represents a unique case as a freestanding NMT catalyzing the terminal iterative methylation of NRPs. These findings not only broaden our understanding of NMT activities but also highlight the diverse roles these enzymes play in modifying and enhancing the bioactivity of NRPs.

Keywords : N-methyltransferases; Nonribosomal peptides; *Leucinostatins*; *Purpureocillium lilacinum*; Antibiotics.

M2

Characterizing the potential of wild yeasts as bioprotective agents in brewing

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Abstract :

The ever-rising demand for new and innovative beers has been motivating brewers to provide products with distinct flavors and properties. Some beer styles are prone to contamination. The use of bioprotective cultures is preferable to the addition of chemical agents to mitigate contamination based on the customer's demand for natural products. As some yeasts are known to have antagonistic activity against beer spoilage microorganisms, this study aimed to identify promising strains within a collection of 640 wild yeasts isolated from different substrates (insect, fruits, flowers, trees, soil) across Quebec and Ontario. In a high-throughput spot-on-lawn assay, 307 strains showed antagonistic activity toward at least one brewery contaminant (*Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus damnosus*, *Brettanomyces clausenii*, *Brettanomyces bruxellensis*, *Pichia membranifaciens*, *Pichia fermentans*, *Pichia kluyveri*, *Pichia kudriavzevii*, and *Gluconobacter oxydans*). The rate of antagonistic activity was linked to the strain isolation substrate (Likelihood test, Pvalue).

Keywords : Bioprospection; Fermentation; Antimicrobial; Antifungal.

In vitro investigation of phage targeting *E. coli* K1 in pregnant donors' intestinal microbiota

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Abstract :

Escherichia coli K1 is a leading cause of human neonatal meningitis, with mortality rates of 10% in developed and 40-50% in developing countries. Among the survivors, 50% experience devastating neurological sequelae and permanent disabilities. The asymptomatic carriage of these strains in the maternal intestinal microbiota constitutes a risk of vertical transmission to the infant at birth. Phage therapy is a promising alternative to antibiotics and previous work has enabled the selection of the phage vB_EcoP_K1_ULINTec4, active against avian and human O18:K1 strains. This work aimed to evaluate the efficacy of phage therapy against *E. coli* O18:K1 in an intestinal environment and its impact on the intestinal microbiota. For this purpose, three independent experiments were conducted on the SHIME® system, the first one with only the phage vB_EcoP_K1_ULINTec4, the second experiment with only *E. coli* K1 and the last experiment with both *E. coli* K1 and the phage. Microbiota monitoring was performed using metagenetics, qPCR, SCFA analysis and the induction of AhR. The results showed that phage vB_EcoP_K1_ULINTec4, inoculated alone, was progressively cleared by the system and replicates in the presence of its host. *E. coli* K1 persisted in the microbiota but decreased in the presence of the phage. The impact on the microbiota was revealed to be donor dependent, and the bacterial populations were not dramatically affected by vB_K1_ULINTec4, either alone or with its host. In conclusion, these experiments showed that the phage was able to infect the *E. coli* K1 in the system but did not completely eliminate the bacterial load. This research was funded by the Walloon Public Service, BLOWIN (Health Cluster of Wallonia, Belgium) project: Inteliphages.

Keywords : Phage therapy; *Escherichia coli* K1; Intestinal microbiota; SHIME; *Galleria mellonella*.

K1 Capsule-dependent phage-driven evolution in *Escherichia coli* leading to phage resistance and biofilm production

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Abstract :

E. coli K1 is involved in several types of human infections, including meningitis, urinary tract infections and bloodstream infections. The understanding of the bacterial resistance mechanisms to phages has implications for the development of phage-based therapies. The objective of this study was to investigate the resistance of *E. coli* K1 isolates to ULINTec4, a K1-dependent bacteriophage. Resistant bacterial colonies were isolated from an avian pathogenic *E. coli* strain APEC 45 and the human strain C5, both previously exposed to ULINTec4. After confirming their resistance and stability, genomic analysis was carried out and several parameters were evaluated, such as growth capacity, phage adsorption, phenotypic impact at capsular level and virulence in the in vivo *Galleria mellonella* model. One of the resistant isolates exhibited a significantly slower growth rate suggesting the presence of a resistance mechanism altering its fitness. Comparative genomic analysis revealed insertion sequences at various capsular gene sites. Adsorption of the ULINTec4 phage was reduced on all resistant isolates. In addition, antigenic tests targeting the K1 capsule showed a very low positive reaction compared to the control. Nevertheless, microscopic images revealed the presence of capsules and a clustered organization of resistant strains. In the *G. mellonella* model, larvae infected with phage-resistant strains showed better survival rates than larvae infected with phage-sensitive strains. In conclusion, a phage resistance mechanism was detected at the genomic level, have an impact on capsular expression and was able to decrease the virulence of *E. coli* K1 in-vivo. The project was financially supported by Wallonia in the framework of the call for projects organised by BioWin competitiveness cluster (Project Inteliphages).

Keywords : Phage resistance; *Escherichia coli* K1; Genome analysis; *Galleria mellonella*; Biofilm.

Multi-sourced potential bacteriocin-producing enterococci: antimicrobial, safety evaluation, and potential probiotic properties

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Abstract :

Introduction: Bacteriocins are globally regarded as natural and sustainable antimicrobial substances for broad application in health and agrifood systems for pathogen control and microbiome modulation. Objectives: In this study, we evaluated the antimicrobial, safety, and probiotic potential of bacteriocin-producing enterococci from multiple sources.

Methods: A total of 524 enterococci previously isolated from humans, rhizosphere, wastewater, animals (dog, wild rabbit, rat, stock, pig, and vulture), and farm environment and identified through MALDI-TOF mass spectrometry and 16S rRNA sequencing were evaluated for antimicrobial activity against broad range pathogens through spot-on-lawn and agar-well diffusion techniques. Enterococci that demonstrated antimicrobial activity were further evaluated for safety (haemolytic activity and antimicrobial resistance) and probiotic properties, including bile salt and phenol tolerance, survivability in simulated gastric juice, hydrophobicity, and auto-and co-aggregation.

Results: Out of the 524 multi-sourced enterococci examined, 18 strains and their cell-free supernatants (CFS) showed broad-spectrum activity against pathogens of public health importance, including *Enterococcus faecalis*, *E. faecium*, *E. cecorum*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Micrococcus luteus*, *Bacillus cereus*, *Clostridium perfringens*, and *Salmonella spp.* The CFS of the majority of the 18 enterococci were stable at 100°C for 20 min and displayed inhibitory activity at neutral pH and after treatment with catalase. The selected 18 enterococcal strains were non-haemolytic and showed varying auto-and co-aggregation and hydrophobicity abilities ranging from 32.93 to 96.31%, tolerance to phenol, bile, and simulated gastric juice with viable counts of 3.0 to 6.0 log cfu/mL. The antibiotic susceptibility test showed 44.44 and 16.67% phenotypic resistance of the 18 enterococcal strains to ciprofloxacin and penicillin, with multiple antibiotic resistance indices below 0.2.

Conclusions: The results suggest that the selected enterococci have the potential to be used in pathogen control, bio-preservation, and microbiome modulation in agrifood and health systems.

Keywords : Enterococci; Bacteriocin; Pathogen; Antimicrobial; Health; Agrifood.

Exploring the biological activities of *Pseudomonas*-derived biocontrol compounds on the wheat-*Zymoseptoria tritici* pathosystem

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Abstract :

Zymoseptoria tritici is a hemibiotrophic fungal phytopathogen which is responsible for one of the most concerning foliar diseases on wheat, Septoria tritici blotch. This disease leads to disastrous yield reductions which can reach up to 50% during severe epidemics. The methods currently used to control this disease rely mainly on the use of chemical fungicides. However, because of the increasing concerns about the potential impacts of conventional phytosanitary products on human and environmental health, new agroecological protection tools have to be developed. One of the most promising alternatives is the use of microbials, and the large range of biomolecules they are able to synthesize, as biocontrol products. Here, we summarize some of the results displayed by crude extracts, metabolites (cyclic lipodepsipeptides (CLPs), rhamnolipids (RLs) and siderophores) as well as bioinspired molecules (rhamnolipids), obtained from *Pseudomonas sp.*, on the wheat-*Z. tritici* pathosystem. Firstly, the direct effect of all of these compounds against *Z. tritici* was assessed and the most active ones were selected for further experiments. Whereas CLPs and siderophores did not present significant antifungal activity in our conditions, they could display other benefits for wheat plants. Culture extracts of *Pseudomonas syringae* as well as some RLs exhibited significant fungal growth inhibition and had their protection efficacy evaluated in greenhouse conditions. In particular, the biological activities of 19 RLs on the wheat-*Z. tritici* were assessed. Among them, mono-RLs with either ether or ester links and a fatty acid tail composed of 12 carbons were the most promising ones. More specifically, the Rh-Est-C12 was found to be the most active compound, activating plant ROS detoxifying pathway and reducing disease symptoms by up to 79% in planta. This study provides new insights into the biocontrol activity of *Pseudomonas*-derived compounds against *Z. tritici*.

Keywords : Wheat; *Zymoseptoria tritici*; Biocontrol; *Pseudomonas*; Rhamnolipids.

Microbulbifer-derived pigments: A novel solution for treating infections caused by pathogenic bacteria

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Abstract :

Antimicrobial agents, such as antibiotics, are essential for treating infections caused by pathogenic bacteria. However, the alarming rise of bacterial resistance poses a significant threat, with few molecules developed to counter this challenge. Fortunately, marine environments offer vast potential with unexplored bioactive compounds such as halogenated furanones, pigments, and antimicrobial peptides. This project focuses on exploring the antibiofilm properties of natural pigments derived from specific marine bacteria isolated from the Atlantic coast (La Rochelle, France). We initially created a natural pigment library, isolating 11 marine bacteria, one specie (Microbulbifer) which exhibited notable characteristics caught our attention. Eco-friendly extraction and purification methods will be employed to produce this pigment, and its antimicrobial effect was initially evaluated against several Staphylococcus species. The antimicrobial effect was tested first on Petri dish but also during 48 hours by using liquid culture of the target strain. Subsequently, its antibiofilm activity against *Bacillus* and/or *Pseudomonas* species will be assessed, along with its anticancer activity using cancer cell lines like MDA-MB-231 (breast cancer) or A2058 (melanoma). Additionally, we aim to characterize the molecules present in this extract with precise identification using mass spectrometry techniques. In conclusion, this project represents an innovative and promising approach to tackling critical public health challenges. We plan to discover new bioactive molecules of marine origin, develop new therapies to combat multi-resistant bacterial infections and cancer, and make efficient use of the marine resources of the Charente-Maritime region.

Keywords : Antimicrobial, Biofilm, Marine-derived, Pigments, Antibiotic resistance.

M8

A novel endolysin based approach for control of the wine microbiota

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Abstract :

Grapes are the largest fruit crop in the world and in 2023 alone, the European Union produced 15,9 billion litres of wine. Winemaking is a complex procedure considering many factors, including those involving the resident wine microbiota. which consists of *Oenococcus oeni* and a variety of other lactic acid bacteria. Some members of the wine microbiota primarily have a positive influence on the final product, although others cause organoleptic defects. Therefore, control of spoiling bacteria in wine is of great importance and currently sulphite addition is among the most commonly used strategies. In line with the global desire to reduce the use of chemicals in food products, the search for alternatives is ongoing. One promising alternative is the application of endolysins which are phage-encoded hydrolytic enzymes that degrade the peptidoglycan layer of the bacterial cell wall. We present a strategy to exploit phage-derived endolysins as antimicrobials to prevent wine spoilage. Using in silico analysis, the rich collection of wine related bacteria at the institute is searched for the presence of endolysins and their diversity will be determined. Candidate endolysins will be chosen, purified and used to study their potential for in vino application.

Keywords : Endolysin, Phage, Wine, Antimicrobial, Enzybiotics.

Anti-microbial potential of micro-organisms isolated from *Curcuma longa*

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Abstract :

Around the world, phytopathogens are responsible of around 20 to 40% of losses in agricultural production. Development and application of biological control agents allow the control of plant diseases but with lower environmental and financial impacts than synthetic phytosanitary products. Production of new antimicrobial biomolecules represents opportunities in biocontrol field regarding health and economic challenges. In this specific context, external and endophytic flora were isolated from fresh *Curcuma longa* rhizomes from Cameroon to study the antimicrobial potential of these microorganisms. Identification by mass-spectrometry (MALDI-ToF-MS) revealed a yeast strain (*Candida sp.*), several fungal strains including *Penicillium sp.* and *Fusarium sp.* and above all, 64% of bacterial strains. The majority of this bacterial population was represented by *Bacillus sp.* and *Pseudomonas sp.* genera. A first stage of screening the antimicrobial activities of bacterial strains enabled us to select some of them, and then to study in greater depth the mode of action responsible for these activities. A production of various interesting lipopeptides (fengycin, surfactin, iturin, bacillomycin) and polyketides (bacillaene, difficidin, macrolactin) were characterized from the culture supernatants from these strains. Moreover, two *Bacillus sp.* strains (*Bacillus siamensis*, *Bacillus velezensis*) culture supernatants showed antimicrobial activities against a range of pathogen targets as *Agrobacterium tumefaciens* C58 or *Pseudomonas aeruginosa* ATCC27853. Finally, these actives strains isolated from the *Curcuma longa* plant could potentially be exploited in biocontrol to fight phytopathogens.

Keywords : Phytopathogens; Antimicrobial; *Curcuma longa*; Lipopeptides; *Bacillus*.

Diversity of microcins within *Enterobacteriaceae* isolates from poultry

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Abstract :

Microcins are bacteriocins produced by *Enterobacteriaceae*. They contribute to shaping the gut microbiome and constitute an attractive alternative to antibiotics, due to their activity against multidrug-resistant (MDR) *Enterobacteriaceae* and narrow spectrum of activity [1,2]. Genome mining shows that this family is still under-explored [3,4]. To evaluate the diversity and distribution of microcins and potentially discover new representatives, 38 *Enterobacteriaceae* strains isolated from broiler chicken were evaluated for activity against MDR strains, presence of microcin biosynthetic gene clusters (BGCs) and production. Antibacterial activity against at least one MDR strain was observed for 21 strains. Genome analysis revealed BGCs of class I microcins ($M_w \leq 5$ kDa, post-translationally modified), the lasso peptide MccJ25, the nucleotide peptide MccC and class II microcins (5 kDa $< M_w < 10$ kDa, siderophore peptides or unmodified peptides), the unmodified MccL and MccV. One genome harbored the MccJ25 BGC and a BGC corresponding to a new class II microcin. Certain strains potentially produced two or three microcins. LC-MS analysis of the supernatant extracts revealed the production of all the class I microcins predicted. The detection of class II microcins was more difficult. Sample preparation and analysis were optimized using a strain producing the well-known siderophore microcin E492 as control. This study shows that *Enterobacteriaceae* isolated from poultry display attractive activities against MDR strains and a high distribution of microcin biosynthetic machineries, which can be accumulated in one strain. The isolated collection represents a rich source of new microcins and an asset for exploiting strains that produce combinations of microcins and examining the microbial ecology mediated by microcins in the gut microbiota.

References [1] Telhig et al., *Front. Microbiol.* 11:586433 (2020) [2] Telhig et al., *Microbiol. Spectr.* 10:e0275221 (2022) [3] Li et al., *J. Agric. Food Chem.* 69:8758 (2021) [4] Cole et al., *Appl. Environ. Microbiol.* 88:e0148622 (2022)

Keywords : Bacteriocins; Microcins; Genome mining; Peptidomics.

Evaluation of potential bacteriocin-producing bacteria recovered from soil. A holistic OneHealth strategy against multidrug-resistant pathogens and for food-industry applications

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Abstract :

Introduction/Objective: Soils are reservoirs for a wide variety of bacteria, that coexist and compete for access to better nutrient sources by producing bacteriocins as a defense mechanism to a hostile ecosystem. These antimicrobial peptides would be of great interest for biomedical and food-industry applications. Therefore, the objective of this study was to evaluate the antimicrobial-activity (AA) of soil bacteria against a wide range of relevant pathogens.

Material and Methods: Fifty-eight isolates recovered from soil samples were selected as potential bacteriocin-producing bacteria (P-Bac+) from a broad screening performed in La Rioja region during 2019-2023, linked to the “MicroMundo project”. They were identified by MALDI-TOF. The AA was confirmed by the spot-on-lawn method against 15 indicator-bacteria, including multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Listeria monocytogenes*, among other relevant pathogens. Antimicrobial-susceptibility testing of P-Bac+ was performed by the disk-diffusion method and β -hemolytic activity was also tested. Selected P-Bac+ strains were submitted to whole-genome-sequencing (WGS) for genomic characterization.

Results: Fifty of the 58 isolates evaluated showed AA against at least one of the 15 indicator-bacteria tested and were considered as P-Bac+. They were of 13 genera and 25 species (genera(number isolates)): *Bacillus*(30), *Pseudomonas*(8), *Brevibacillus*(6), *Paenibacillus*(4), *Streptomyces*(2), *Staphylococcus*(1), *Advenella*(1), *Arthrobacter*(1), *Brachybacterium*(1), *Klebsiella*(1), *Lysinibacillus*(1), *Microbacterium*(1), and *Peribacillus*(1). Among P-Bac+ isolates, 51.4% were susceptible to all antibiotics tested and 67% lacked β -hemolytic activity. The *Brevibacillus laterosporus* strain MM3 revealed AA against 8/15 indicators tested with strong inhibitory activity against MRSA and *L. monocytogenes*. Ten isolates [*Staphylococcus hominis*/(1), *Bacillus pumilus/safensis/altitudinis/mycooides*/(5), *Brevibacillus laterosporus*(2), *Paenibacillus apiarius*(1) and *Pseudomonas kilonensis*(1)] were selected for WGS characterization (in process).

Conclusions: Soils are reservoirs of bacteriocin-producing bacteria with antimicrobial-activity against relevant pathogens, which is of great interest for biomedical and food-industry applications.

Keywords : Bacteriocins; Soil; Antimicrobial; Pathogens.