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Model microbial communities for biocellulose production: dynamics, labour division and functionalities

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Bacterial cellulose (BC) is a biopolymer useful for several food and biomedical applications. BC production suffers from several constraints (strain-dependent, variability, high costs) and, therefore, strategies to improve BC yield is of practical relevance.

In the Project SynBioCell, 12 model microbial communities (MMC) were constructed by assembling, through a combinatorial approach, 4 acetic acid bacteria (AAB, core strains and BC-producers), 3 lactic acid bacteria (LAB, helper) and 3 yeasts (Y, helper), to boost BC yield, compared to individual AAB strains. The best MMC (including 2 AAB and 3 LAB) was select and re-combined to evaluate the microbial dynamics, cell compartmentalisation and strain functionalities during BC production (plate counting, BC yield, 16S rRNA-based qPCR, qPCR on cellulose sintasi complex, ^{1}H-NMR spectroscopy). Metatranscriptomic analyses were also performed to investigate the metabolic interaction of MMCs members.

The composition of MMCs significantly affect the BC production and, generally, LAB stimulated the BC yield. A good correlation was found for strain occurrence detected with plate counting and *16S rRNA* gene. qPCR confirmed the overexpression of CS genes in LAB-containing consortia. Metatranscriptomic data revealed that AAB functionalities significantly changed in MMC compared to the single AAB cultures, due to the shift in metabolic pathway stimulated by the presence of LAB. Our results showed that targeted MMCs could be an efficient machinery to improve BC production and provided further insights on metabolic networks and dynamics of microbial consortia.

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Salt-enhanced IAA production in *Vreelandella titanicae*: a stress-responsive for sustainable agriculture in saline soils

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Plant Growth-Promoting Rhizobacteria (PGPR) can play a crucial role in promoting plant growth under stressful conditions, such as in degraded soils. Soil salinization is becoming a rapidly expanding phenomenon, especially in Mediterranean countries, and represents a global threat to agricultural productivity. In this context, the isolation of halotolerant/halophilic PGPR and their characterization are the basis of a biotechnological and sustainable approach to addressing these problems.

We isolated the halotolerant bacterium *Vreelandella titanicae* from quinoa rhizosphere and characterized it for its PGP traits. The strain showed high indole-3-acetic acid (IAA) production, a key phytohormone involved in plant growth and development. Several studies on salt-tolerant bacteria reported the effects of different chemical-physical parameters on IAA production, however, the role of salt remains unclear. To address this gap, we characterized *V. titanicae* at genomic, metabolic and physiologic level and investigated NaCl-regulation of hormone production. Growth kinetic studies were performed under increasing salt concentrations, and IAA was quantified over time. Gene expression analysis was conducted focusing on the key genes involved in tryptophan-dependent IAA biosynthetic pathway. Increasing salinity reduced biomass but enhanced IAA production, reaching the greatest levels at 1.0 M NaCl. Expression analyses highlighted that salt positively regulated genes involved in the indole-3-acetaldehyde (IAAld) pathway, suggesting that osmotic stress activates specific metabolic ways of IAA production.

Our results demonstrate that IAA production in *V. titanicae* is a stress-responsive trait and provides further insights into its biosynthetic pathway and regulation, while, also identifying this bacterium as a promising PGPR candidate for salinized soils.

Evaluation of the anti-tubercular activity of *Kielmeyera membranacea* constituents

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Tuberculosis remains the leading cause of mortality from a single infectious agent worldwide, with 1.25 million deaths and 13.4 million new cases in 2023. The emergence of multidrug-resistant and extensive drug-resistant strains underscores the urgent need for new therapeutic alternatives. Natural products represent a valuable source of bioactive compounds, historically providing a significant contribution to antimicrobial drug discovery.

Kielmeyera membranacea (*Calophyllaceae*) has previously demonstrated antitubercular activity. This study aimed to identify the compounds responsible for this activity and to evaluate the influence of climatic factors on their production. Plant material was collected from four restinga regions across all seasons. Antitubercular activity was assessed using the Resazurin Microtiter Assay against *Mycobacterium tuberculosis* H37Rv, revealing MIC_{90} the lowest value as 125 µg/mL, with variation across regions suggesting a influence of climatic factors on bioactive compound production.

Phytochemical analysis identified the biflavonoid podocarpusflavone A (PCFA) as one of the major constituents. PCFA exhibited synergistic activity with isoniazid, significantly inhibiting intracellular bacterial growth in THP-1 macrophages. *In silico* analysis suggested protein tyrosine phosphatase B (PtpB) as a potential target. To investigate this mechanism, *ptpB*-overexpressing strain was evaluated in infection assays. Treatment with PCFA (128 µM) and isoniazid (0.2 µM) resulted in a ~10-fold increase in CFU/mL compared to H37Rv strain suggesting that PtpB overexpression reduced susceptibility to the PCFA/isoniazid combination. Additionally, IL-6 and caspase-3 levels were significantly modulated, indicating that PCFA probably modulates host immune defense mechanism.

These findings highlight PCFA as a promising antitubercular candidate with immunomodulatory properties and potential for combination therapy.

Modulation of glycolysis and homolactic fermentation of *Streptococcus thermophilus* energetically discharged cells: dissecting the role of transcription and post-translational modifications

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S. thermophilus metabolizes lactose via homolactic fermentation. Lactose enters the cell through LacS, is hydrolyzed by β-galactosidase into glucose and galactose, the galactose is exported, and the glucose is converted to lactic acid via glycolysis. To shed light on the regulatory network of glycolysis and homolactic fermentation, cells harvested in the exponential growth phase were divided into aliquots and subjected or not to an ATP and glucose depletion treatment, in presence and absence of rifamycin and chloramphenicol to block transcription and translation. The resulting ATP or glucose depleted biomasses (EdCs) were subjected to comparative metatranscriptomics and metaproteomics analysis, and quantification of (p)ppGpp. The EdCs phenotype showed the lowest levels of (p)ppGpp and an up-regulation of the S10 ribosomal protein gene cluster and purine biosynthesis genes, consistent with the regulatory scenario headed by the stringent response. The metabolic behaviour of those biomasses was then evaluated by activating them with lactose, lactose and urea, in presence or in absence of glycolytic inhibitors. EdCs activated with lactose behaved as biomasses treated with glycolytic inhibitors, displaying no production of L-lactic acid, while exhibiting a higher lactose capacity compared to their control. Data analysis revealed transcriptional regulation of genes and post-translational modification of proteins involved in glycolysis correlated with the yield of glycolysis and lactose intake capacity. Proteomic analysis showed ATP and glucose depletion significantly alters protein lactylation profiles, indicating that addition and removal of lactyl groups on lysine residues are regulated at post-translational level by acetyltransferases and deacetylases.

Display of an influenza virus multi-epitope on *B. subtilis* spores

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Bacterial spores are promising tools for the mucosal delivery for the development of new oral/nasal vaccines, offering stability, safety, and the ability to stimulate both humoral and cellular immune responses. This project explores the use of probiotic strains of intestinal origin of *B. subtilis* to display influenza epitopes and test them as mucosal vaccine on animal models. Compared to laboratory strains, probiotic strains may provide enhanced performance due to their adaptation to the gastrointestinal environment and to the positive effects of probiotics on the efficacy of mucosal immunizations. A probiotic strain of intestinal origin of *B. subtilis,* MV24*,* was selected according to the EFSA guidelines for safety. A chimeric Influenza A multi-epitope (ME-IVA) was designed, using IEDB, CALIBER and BepiPred 3.0 epitope prediction tools and a synthetic gene coding for ME-IVA produced. Specifically, the construct includes conserved T-cell epitopes from Influenza A Nucleoprotein (NP) and Matrix Protein 1 (M1), along with B-cell epitopes from Hemagglutinin (HA), Neuraminidase (NA), and the M2 ectodomain (M2e). These domains were connected with appropriate linkers to optimize processing, folding, and immunogenicity. The final sequence was codon-optimized for *E. coli* expression, allowing efficient overexpression and purification via affinity chromatography. The purified protein is being used for non-recombinant surface display through adsorption onto purified MV24 spores. In parallel, recombinant approaches are being developed by generating gene fusions between the ME-IVA sequence and spore coat proteins (CotB and CotC), enabling antigen display directly on the spore surface. Ongoing experiments are optimizing protein expression and surface display, which will allow future purification and immunological studies *in vivo*. This work focuses on developing a safe and stable probiotic spore-based platform for broad, cross-protective influenza vaccines.