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Integrating GC–MS and customized MOX gas sensors for VOC-based discrimination of bacteria: towards applications in sustainable agriculture and food safety

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Abstract

Climate change is increasingly threatening agricultural productivity, particularly in Mediterranean environments where crops are exposed to recurrent drought and soil degradation. In this context, plant growth-promoting bacteria (PGPB), such as *Streptomyces violaceoruber* and *Kocuria rhizophila*, represent a promising sustainable strategy to enhance plant resilience to abiotic stresses. Microbial and plant metabolic activities are closely related to the emission of volatile organic compounds (VOCs), which can serve as non-invasive biomarkers of physiological status and stress conditions. In this study, we combined solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME/GC-MS) and custom-developed metal oxide (MOX) gas sensors to characterize and monitor VOC profiles from beneficial and pathogenic microorganisms. SPME/GC-MS analyses enabled the identification of species-specific volatilomic signatures in pure cultures of PGPB, revealing putative volatile markers associated with different growth stages. Notably, compounds such as geosmin and 2-methylisoborneol were detected in *S. violaceoruber* cultures and in soil samples, confirming the applicability of the method to real and complex environmental matrices. In parallel, MOX gas sensor arrays coupled with multivariate statistical analysis demonstrated the possibility to discriminate between different bacterial species and subspecies in a rapid and non-invasive manner. This approach was further validated on biological samples artificially contaminated with *Salmonella enterica*, highlighting distinct volatilomic fingerprints. Overall, our results demonstrate that the integration of GC-MS and MOX sensor technologies provides a robust and complementary platform for VOC-based microbial discrimination. This approach represents a promising tool for applications ranging from precision agriculture—through monitoring of PGPB activity and plant health—to food safety diagnostics, paving the way for real-time, in field monitoring systems.

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Unravelling antimicrobial resistance spread within populations trough integrated metagenomic and culturomic analyses of wastewaters.

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Antimicrobial resistance (AMR), occurring when pathogens evolve to withstand antimicrobial drugs, is making infections more difficult to treat and can be regarded as a "silent pandemic" that may cause up to 10 million deaths annually by 2050. In this scenario, wastewater-based epidemiology (WBE) may contribute to the surveillance of AMR diffusion and evolution. We implemented WBE to investigate AMR in specific settings, such as those characterized by high antibiotic usage (e.g. hospitals) and those representative of a young and healthy population (e.g. universities), in comparison with the whole community, by analysing different sewage sources from the same urban environment. ESBL-producing *Escherichia coli* prevalence was similar regardless the sewage source, coherently with the widespread use of cephalosporins. On the contrary, vancomycin resistant *Enterococci* (VRE), among major causes of healthcare-associated infections, were higher in wastewater from the hospital, where this antibiotic is manly used. Whole genome sequencing and phenotypic characterization indicated the occurrence of peculiar resistance features of hospital-derived strains. Quantification by qPCR/dPCR of selected antimicrobial resistant genes (ARGs), together with metagenomic-based high-throughput profiling of ARGs, showed population-specific resistance patterns, with a higher abundance of ARGs related to clinically relevant resistances in sewages representing the hospitalized population. Taken together these results indicate how the nosocomial environment, characterized by a large use of antibiotics, may represent a hot-spot for AMR. The application of WBE to specific settings provides high-resolution clues on the dimension of AMR within specific sub-populations, contributing to the definition of public-health interventions to combat antimicrobial resistance.

Lactococcus lactis postbiotics on human glioblastoma cell lines: _in vitro_ evaluation of antitumor activity.

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In recent years, probiotics have attracted increasing attention in the medical, pharmaceutical, and food fields due to their well-documented health benefits. This growing interest has driven scientific research toward the identification and characterization of novel probiotic strains. We established a collection of 50 lactic acid bacteria (LAB) isolated from dairy products, including *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum*, and *Lactobacillus helveticus*. Following a preliminary screening of probiotic-related properties, such as tolerance to acidic conditions and bile salts, antibiotic resistance profiles, cell surface hydrophobicity, autoaggregation ability, and production of antimicrobial compounds, the best-performing strains were selected for further investigation of their potential anticancer properties. In our study we focused on the potential antitumor activity of postbiotics (functional fermentation compounds) produced by three selected high-performing *Lactococcus lactis* subsp. *lactis* strains on human glioblastoma cell lines. Cell viability assays, including MTT and Trypan Blue exclusion tests, showed a significant reduction in cell proliferation following treatment. Flow cytometry analysis confirmed these findings, demonstrating cell cycle arrest in treated cells. Furthermore, postbiotic treatment significantly inhibited cancer-related processes such as wound healing and cell migration. On the other hand, primary astrocytes viability and the blood-brain barrier (BBB) integrity were not impaired, suggesting a selective effect of postbiotics on proliferating-undifferentiated cells. This preliminary study highlights, for the first time, the potential anticancer properties of postbiotics from some *L. lactis* strains on human glioblastoma cell lines.

From transcriptomic profiling to engineering target selection in *Haloferax mediterranei* for PHBV and bacterioruberin production

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Haloferax mediterranei is a promising archaeal platform for next-generation industrial biotechnology, owing to its natural ability to synthesize both poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), a promising PHA-copolymer, and bacterioruberin, a rare C-50 carotenoid [1]. Since the accumulation of these compounds is promoted by distinct cultivation conditions, understanding how carbon flux is redistributed between the two pathways is essential for rational strain engineering. In this work, transcriptomic profiling was performed on *H. mediterranei* cultivated under different conditions identified by statistical optimization as favoring either PHBV or bacterioruberin production [2]. Differential expression analysis highlighted condition-specific regulatory and metabolic responses linked to carbon flux distribution, precursor supply, and carotenoid biosynthesis.

The integration of transcriptomic data with pathway knowledge enabled the selection of candidate targets for metabolic engineering. For PHBV-oriented strategies, *korAB* and *porAB* were identified as relevant targets associated with propionyl-coA supply, while *cimA* was selected for promoter engineering to further modulate precursor flux. For bacterioruberin-oriented strategies, *lyeJ* emerged as a candidate target from transcriptomic analysis, and promoter engineering of *crtB* and *crtI* was designed to modulate flux through the carotenoid biosynthetic pathway. These results establish a transcriptomics-guided framework for target prioritization in *H. mediterranei* and support ongoing engineering efforts aimed at improving PHBV and bacterioruberin production.

References

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Synthesis and characterization of postbiotics from *Bacillus* spp.: study of functional properties and applications

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ABSTRACT

Despite their widespread use, the clinical efficacy of probiotics is highly variable and often inconsistent. Their functionality, in fact, is often impaired by limited survival, reduced engraftment and decreased metabolic activity during transit through the gastrointestinal tract. This hinders their capacity to exert sustained and biologically meaningful effects within the host. These limitations significantly impair their ability to colonise or transiently integrate into the resident microbiota, thereby restricting stable host–microbe interactions. Consequently, the anticipated health benefits are frequently diminished, transient, or absent, giving rise to significant concerns regarding their reliability as therapeutic interventions. In this context, postbiotics — defined as non-viable microbial cells, their components, or metabolites that confer health benefits — are emerging as a promising alternative. Unlike probiotics, postbiotics do not rely on microbial viability, thereby overcoming challenges related to stability, storage, and gastrointestinal survival. Furthermore, they offer greater safety, standardisation and mechanistic clarity, making them more suitable for precise and reproducible therapeutic applications. These features position postbiotics as a next-generation strategy for microbiome-based interventions. In this study, we focus on *Bacillus*-based postbiotic production using alternative carbon sources, particularly prebiotic substrates such as inulin and fructooligosaccharides. These are not digested by the human host, but can be metabolised by microorganisms, thereby enhancing the production of beneficial metabolites. This combined approach is a promising strategy for overcoming the current limitations of probiotics and supporting the development of applications for gut and skin microbiota.