WEEK OF MICROBIAL TECHNOLOGIES

ABSTRACTS BOOK

7-11 NOVEMBER, 2022
LJUBLJANA, SLOVENIA

Jožef Stefan Institute

SURF BIO

Surfbio project has received funding under the European Union's Horizon 2020 research & Innovation programme under grant agreement N° 952379

BOOK OF ABSTRACTS

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Ljubljana, Slovenia

November 7 – 11, 2022

Book of Abstracts: Week of Microbial Technologies

7 – 11 November, 2022 - Ljubljana, Slovenia

Organising committee: Jožef Stefan Institute, ICCRAM University of Burgos, AXIA Innovation, Wageningen University and Research, Ghent University, Helmholtz-Zentrum Dresden-Rossendorf.

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CONTENT

PREFACE

The Week of Microbial Technologies –MicroTechWeek– was a five-day summit full of project meetings and open public events, such as an industrial workshop, a poster session and a hands-on training, aimed at gaining knowledge on the applications of surface and colloid biology in different industrial sectors. It was jointly organised by the European projects SURFBIO and GREENER.

The main goal of this event was networking between EU projects and stakeholders, sharing applications of surface and colloid biology and planning new initiatives based on microbiology technologies.

This book gathers the contents generated in the SURFBIO industrial workshop and in the poster session. Professionals from international companies and organisations contributed knowledge from different perspectives, creating very fruitful roundtables for the project partners.

SURFBIO Project has received funding under the European Union's Horizon 2020 research & innovation programme under grant agreement Nº 952379.

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ABSTRACTS INDUSTRIAL WORKSHOP

LEGO MICROBES: THE COLLOID BIOLOGY APPROACH TO BUILDING A MICROBIAL COMMUNITY FOR SUCCESSFUL REMEDIATION OF THE ENVIRONMENT

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In the environment, multimicrobial structures such as flocs, mats or biofilms are extremely well spatially organized in terms of microenvironmental conditions and metabolite exchange that allow the establishment of different niches within. However, structures are formed by chance and it is extremely valuable to develop a synthetic approach where structures can be manipulated in a way that are acting as catalytic cores. If they can be tailor assembled and maintained in biotechnological processes such as bioaugmentation during the bioremediation.

To assemble spatially oriented microbial cells within the consortium composed of different cells we need at least four components (4S): (i) Stickiness, a special "glue" to attach one cell to another and then promote their mutual interaction, (ii) Spatial, cells should be spatially oriented according to the design to exchange metabolites, (iii) Stable, structures should not disintegrate in the solution or solid matrix (e.g. soil) after the initiation of microbial growth and (iv) Scalable, a method must be upscaled for the use in the biotechnological systems.

To develop such synthetic structures we treated bacterial cells as colloidal particles to which we can approximate a zeta potential of about -40mV by the Smoluchowski equation. According to DLVO theory if we change the surface potential of one cell it will attach to the cell with opposite charge. By careful manipulation using a top-down approach we were able to prepare special structures enabling the combining of different bacterial cells, including strict anaerobes, forming stable planar or 3D structures. Until now we have successfully applied the “LEGO” approach in the preparation of catalytic structures for organic wastewater processing, metal precipitation from mine tailings, removal of micropollutants, prevention of biocorrosion and revalorisation of lignin wastes from the paper pulp industry.

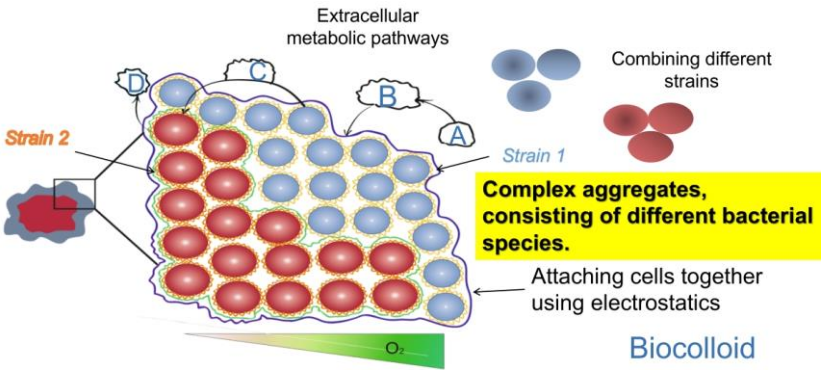

Figure 1. Biocolloid structure synthetically prepared using electrostatic approach to bring different strains in close interaction.

BIOREMEDIATION SYSTEMS EXPLOITING SYNERGIES FOR IMPROVED REMOVAL OF MIXED POLLUTANTS

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BIOSYSMO is a 48-month action that will develop a computationally-assisted framework for designing and optimizing synergistic biosystems combining the required pathways and traits to achieve the most efficient degradation and sequestration of pollutant mixtures. These biosystems will comprise combinations of bacteria, fungi and plants containing the natural or engineered pathways required for pollutants degradation and identified based on a computationally-assisted analysis. BIOSYSMO will take advantage of the high natural microbial diversity by screening samples from polluted sites and locations affected by diffuse pollution to identify natural microorganisms already present and able to metabolize the target pollutants. The search will be expanded to microorganisms previously identified and characterized by applying data mining tools to genomic and metagenomic data available in public repositories.

The construction and optimization of synergistic biosystems will combine approaches based on 1) enhancing plant-microbe (bacteria, fungi) interactions to achieving combinations with improved pollutant uptake and/or degradation; 2) engineering bacteria, for improved degradation and bioaugmentation, and plants (poplar tree), for improved microbial colonization and pollutant uptake; 3) constructing artificial micro-structured consortia into aggregates and biofilms, containing all the required pathways for pollutant removal; and 4) applying bioelectrochemical systems (BES) as stand-alone or in hybrid systems. The different key players will be identified and combined to formulate innovative biosystems with the assistance of genome-scale metabolic (GEM) models for elucidating and simulating the key metabolic pathways. The constructed biosystems will be applied in conventional (phytoremediation, biopile, bioaugmentation) and innovative (BES, hybrid BES-phytoremediation) bioremediation approaches optimized for the treatment of mixtures of pollutants in soil, sediments and waters.

ACTIVE AND INTELLIGENT FOOD PACKAGING

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Active intelligent packaging represents an innovative approach to packaging design that incorporates advanced technologies to actively monitor and interact with packaged products throughout their lifecycle. By integrating various functionalities, such as sensing, communication, and actuation, active intelligent packaging offers numerous benefits, including improved food quality, enhanced safety, by detecting and signaling potential spoilage or contamination extended. Additionally, this technology helps to minimize food waste by providing accurate information about product freshness and shelf life to consumers. This abstract provides an overview of active intelligent packaging, highlighting its key features, applications, and potential future developments. It will be put in the context of relevant projects and infrastructure, including ISO-FOOD, METROFOOD-RI and FoodTraNet.

Active packaging refers to the incorporation of additives into the package with the aim of maintaining or extending the product quality and shelf life. Colloidal systems possess the capability to serve as active coatings, and when applied as colloidal dispersions, they enable extended and regulated release of the active ingredient while ensuring uniform distribution. This is made possible by their colloidal/nano size and the significant surface area-to-volume ratio they exhibit. Chitosan in active packaging will be presented as an example. The results will also show how cold plasma using CO_2 has activated functional groups on the packaging material and reduced migration of active substance from the manufactured films of developed material.

The intelligent systems are those that monitor the condition of packaged food to gather real-time data on product conditions, such as temperature, humidity, and gas levels, ensuring optimal storage and transportation conditions. These sensors can detect potential spoilage or contamination, allowing for timely intervention and reduced product waste. Moreover, active intelligent packaging incorporates communication technologies, such as near-field communication (NFC), RFID, or QR codes, enabling seamless interaction between the product, packaging, and consumers. This connectivity facilitates valuable information exchange, including product origin, handling instructions, nutritional content, and even personalized promotions.

Looking ahead, active intelligent packaging is poised to witness further advancements. Future developments may involve the integration of artificial intelligence (AI) and machine learning algorithms, enabling packaging to adapt autonomously to changing product conditions. Additionally, nanotechnology may play a significant role in enhancing the functionality of active intelligent packaging, enabling more precise and targeted monitoring and control.

In conclusion, active intelligent packaging holds great promise for the food industry. As the technology continues to evolve and become more affordable, it has the potential to revolutionize the way food is packaged, transported, and consumed, leading to a more sustainable and efficient food supply chain.

NATURE-BASED SOLUTIONS: TREATMENT WETLANDS FOR DIFFERENT TYPES OF WASTEWATER

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Nature-based solutions (NBS) imitate natural processes and serve to generate multi-benefit values to the customer or to the local communities. Sustainable solutions developed and performed by Limnos belong to the NBS group of treatment wetlands. They are multi-purpose targeted to wastewater treatment, water retention, and pollution mitigation. They are stimulated as zero-emission and climate-resilient technology.

The main areas of intervention where NBS are best applied for the purpose of this presentation are: wastewater and leachate treatment (constructed wetlands, floating treatment islands), and agricultural run-off mitigation (constructed ecosystems for non-point pollution mitigation).

The most frequent treatment wetland is a constructed wetland (CW) which are biologically engineered wastewater treatment systems that rely on the presence of plants and microorganisms, the interaction of physical, chemical and biological processes, as well as different removal mechanisms. CWs can treat different types of wastewaters: municipal and industrial wastewater, landfill leachate, storm water, agricultural runoff. They efficiently remove suspended solids, organic matter, and nutrients. However, removing heavy metals is not frequent and poses a further experimental opportunity. Each CW consists of a pre-treatment step (septic tank or sedimentation basin) followed by one or more interconnected beds. Remaining challenges under the new legislative demands will be linked to removal of new & emerging pollutants (e.g. microplastics that in the case of the CWs is being retained in the system; lab scale test proved the retention of 99.99% of microplastics are retained in the matrix/sediment of the CW[1]). The strive for increased treatment efficiency supports the development of new matrix materials as well as further application due to the fact that these matrix are lighter which supports the application in urban (grey water treatment) and challenging areas (e.g. mountain cabins).

Co-natural reclamation of landfills increases the resilience of waste infrastructures against climate change (fires caused by droughts and heatwaves support the development of solutions where treated leachate water is reused). Among several NBS applied on the landfill, new innovative floating treatment wetlands can be applied, as is the case in the Life GREEN ADAPT project. This phyto-technology keeps the plant roots permanently in contact with water which enables the removal of pollutants. Floating treatment wetlands (FTW) had been tested for the treatment of landfill leachate at the mesocosm scale, achieving removal efficiencies of 97% for ammonium, and organic matter above 87%[2]. The surface of the FTW required to enhance water

[1] Rozman U., Klun B., Kalčikova G. (2023). Distribution and removal of microplastics in a horizontal sub-surface flow laboratory constructed wetland and their effects on the treatment efficiency. Chemical Engineering Journal, 461, 1385-8947. Doi: https://doi.org/10.1016/j.cej.2023.142076

[2] Saeed T. et al.(2020). Pollutant removal from landfill leachate employing two-stage constructed wetland mesocosms: co-treatment with municipal sewage. Env. Sci. and Poll. Res. 27 28316–2833, doi: https://doi.org/10.1007/s11356-020-09208-y

quality varies greatly. The experience from Xiloga landfill in Spain will develop a new result set in leachate waters treatment.[3]

Mitigation of agricultural run-off by constructed ecosystems positioned in drainage ditches, proved to be an efficient solution for nutrient removal[4]. Constructed ecosystems are varied simple structures of natural materials (sediments, wood, macrophytes). They create conditions for the development of biologically active surfaces where microorganisms increase the nature's self-cleansing capacities and represent a sustainable approach to reducing the pollution load to water resources. The removal rate depends on the pollution loads. The effectiveness of constructed ecosystems was monitored on four (4) field locations in Slovenia and resulted in average 56 % reduction of COD, 50 % reduction of TSS, 67 % reduction of nitrates.

NBS are an efficient technology for preventing or mitigating pollution and supporting natural habitats. The solutions must be adapted to the problem and developed for each particular site.

[3]URL: https://lifegreenadapt.com/ (30.4.2023)

[4]Žvokelj L., Renčelj M., Vogrin M., Brodnik U., Pintar M. (2021). EIP GREKO - Grajeni ekosistemi za blaženje vpliva kmetijstva na okolje oz. zaščito kmetijskih zemljišč. Mišičev vodarski dan., 267-274. URL: https://www.mvd20.com/LETO2021/R36.pdf.

SERS: PUSHING THE LIMITS OF RAMAN DETECTION

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An amplification of the Raman signal is caused by the enhancement of the electric field provided by the SERS substrate. When the incident Raman laser beam strikes the active spot of the SERS substrate, localized surface plasmons are excited. The nanoparticle layer is responsible for this resonant enhancement (Figure1, left). The SERS effect is extremely pronounced because the field enhancement occurs twice: (i) The resonant field enhancement amplifies the intensity of incident Raman laser beam, which excites the Raman modes of the molecules of the analyte and (ii) the resulting enhanced Raman signal is then further amplified by the SERS substrate due to the same resonant effect. An underlying signal enhancing heat sink allows for the utilization of high Raman laser power yielding a proportionally high Raman signal (Figure 1, right).

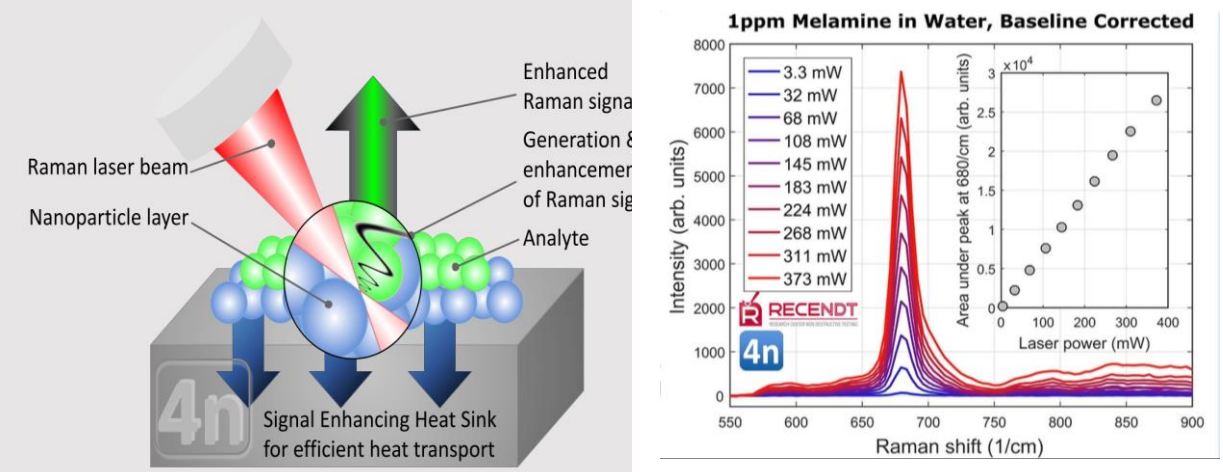

Figure 1: Left: A **SIGNAL ENHANCING HEAT SINK** protects the analyte from overheating by efficient heat removal from the focal spot of the Raman laser beam. Right: Maximum Raman signal is guaranteed at full power from the Raman laser without damaging the substrate nor decomposing the analyte.

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Patent application: Andreas Stingl et. al. SERS substrate comprising nanoparticles, international application number: WO2022266691A1

VERDEQUANT: SUSTAINABLE MANUFACTURING OF HIGH PERFORMANCE NANOMATERIALS AND THEIR APPLICATIONS

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Sustainability of VERDEQUANT processes is defined by 2 basic prerequisites: (i) they are free from the use of fossil based reactants and substitutes them with ones obtained from renewable sources, such as e.g.: plants, dairy products[1] or fungi and use vegan reactants whenever possible and (ii) they are preferably developed in aqueous medium. Water is our first choice solvent (Figure 1).

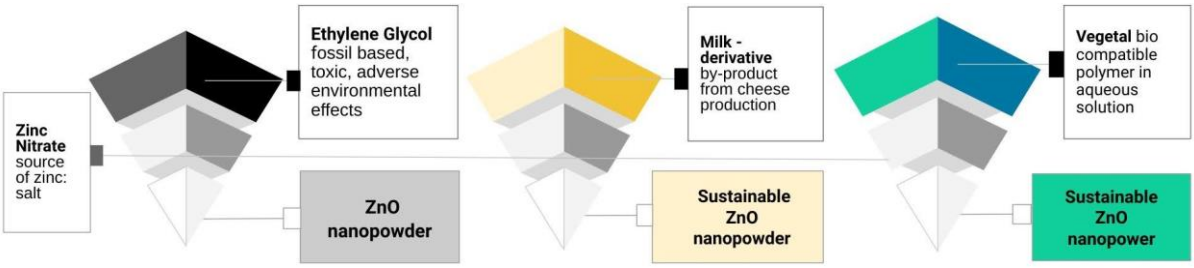

Figure 1: from left to right: (i) traditional sol gel process utilizing Ethylene Glycol (EG) as a gelling agent, (ii) VERDEQUANT process substitutes EG by whey, a byproduct of cheese production, and (iii) a vegetal polymere substitutes whey offering a vegan alternative.

Applications include photovoltaics [2], photocatalysis, cosmetics - sun protectors, antimicrobial agents

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MANUFACTURING ASPECTS OF PHAGE-BASED PRODUCT DEVELOPMENT FROM EARLY STEPS TO PRODUCTION OF CLINICAL TRIAL MATERIAL

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Bacteriophages (phages) are viruses that attach, infect and then replicate in bacterial cells (hosts). Phages are composed of protein capsid that encapsulate DNA or RNA genome and are highly specific to specific bacterial host strains. After attachment to host strain, phage injects its genetic material into the cell and can use host metabolism to replicate, following burst of cell (lysis) to release newly produced phages into the environment. These major characteristics of phages are exploited to use phages as e.g. alternative to antibiotics (known as phage therapy), to alternate human/animal microbiome, as carriers for vaccine (e.g. virus-like particle (VLP)), cosmetics, bacterial contamination control etc. Therefore, there is an increased need for production of various phages of quantity and quality suitable for intended application.

To move candidate phage from research level to end use/user there are a number of requirements that need to be understood/developed. Production bacterial host and phage candidate must be carefully selected to minimize potential hazard and achieve efficacy of final product for end user (e.g. as human therapeutic product in clinical trials) by (i) selecting phages/host with minimal/no presence of undesirable genes; (ii) developing process to be able to achieve high production yields and (iii) selecting phage with suitable efficacy and stability. As phages are mostly produced/propagated in targeted pathogenic bacteria, the residual bacterial impurities (proteins, genetic material) in a final product must be removed during production to a level required for intended application to ensure a safe product. Therefore, upstream and downstream production process must be carefully developed. In addition, final product formulation must be developed e.g. in form of capsules, liquid, dry powder and must ensure a reasonable stability of intact phage particles (product shelf life). To understand final product as well as all production activities development of state-of-the-art analytical methods is essential and should be developed in parallel to all production activities.

JAFRAL is a Contract Manufacturing Organization (CMO) & Contract Research Organization (CRO) with its primary focus on production of bacteriophages (phages) as human therapeutics (investigational medicinal products), as well as for food industry, cosmetics and veterinary use. JAFRAL offer services for the development of production processes, development of analytical methods to support all production processes and routine manufacturing of phage-based products under different certifications (biotechnological products, advance therapy medicinal product).

ECOTOXICOLOGY ASSESSMENTS IN THE DIAGONAL PROJECT: NANOPARTICLES TOXICOKINETICS AND TOXICODYNAMICS IN BIOFILMS

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DIAGONAL is a Research and innovation action that aims to address existing gaps at risk assessment, risk management and risk governance levels, providing new knowledge on multicomponent nanomaterials and high aspect ratio nanomaterials risk, considering the interaction amongst their components and with the environment, as well as their release and fate. Ultimately, the obtained results will serve as basis to provide adapted or novel risk management guidelines, ready to use Safe by Design tools and strategies to increase nanomaterials safety, including Sustainable-by-Design considerations, and recommendations for risk governance. Seven industrial demonstrators, which are producers of either nanomaterials or nanoenabled products, participate in DIGONAL.

In order to obtain results to increase the knowledge and understand the consequences of the exposure to MCNM and HARNs, toxicokinetic and toxicodymanic experiments are conducted in environmentally relevant species. The analysis of both parameters can provide information on the bioaccumulation of pollutants over time as well as on the toxic effects of the accumulated concentration in the organism. Among the different models selected in the project to perform environmental research, biofilms were included since these structures are considered good bioindicators due to their ability to respond rapidly to environmental stress. Considering that there is no OECD validated protocol available yet for biofilms to study toxicokinetic (transfer mechanisms) and toxicodynamic (effects) of xenobiotics, the steps followed in the development of a protocol to analyse both parameters are described in this presentation. Thus, the selection of the bacterial species (*Pseudomonas putida*), and the experimental conditions tested to carry out toxicokinetics and toxidynamics studies (using ROS production as toxic effect under study) are presented.

PARTICIPATORY ENVIRONMENTAL MONITORING AND SMART CITIES - ROOM FOR MICROBIAL TECHNOLOGIES?

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Participatory approaches as part of environmental monitoring are on the rise, often in the context of smart cities and focusing on "classic" challenges in urban environments such as air pollution, noise or thermal stress. This contribution provides a conceptual overview of this field in the light of the development of new sensor technologies and supporting ICT tools, and raises the question of the role of microbial technologies in this context.

It presents examples of the use of new sensor technologies in real-life applications within a wide range of past and ongoing projects, and in a variety of contexts - from usability testing from the perspective of end-users, to determining exposure to urban stressors at the individual level, to environmental epidemiology, and as a support to planning city-scale and personal interventions. The role of researchers on the one hand and citizens on the other is further discussed, as well as the different levels and possibilities of involvement of the latter in project activities in the light of their motivation, interest and expected benefits. Based on examples from the literature, the role of participants in microbial-based citizen-science projects is discussed and some technological challenges for an even more active involvement of lay people in the respective research are highlighted.

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MICROBIAL SOLUTIONS FOR SUSTAINABLE AGRICULTURE

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An advance in microbiome technology and computational biological analyzes allowed evaluation of community composition, potential function, and activity of the selected strains as co-formulants in the phytobiome. Our goal was to develop an innovative blend of microbial and mineral components that would significantly increase the conversion rate and yield of a variety of crops in different soil types, thereby improving the overall performance and sustainability of crop production in general, including organic farming. The ultimate challenge we have identified is to formulate a range of environmentally friendly and health safe bio-based blends, highly efficient biofertilizers with pesticide properties that are not too technologically complex to produce in scalable quantities and consequently available at acceptable cost with solid market and commercialization potential. To this end, we have developed a mixture of compost and biochar as an organo-mineral component that is substituted by biological components of selected bacteria and algae that act simultaneously as fertilizer and pesticide. These components have positive effects by themselves, but in combination they provide a multiple synergistic effect. And the synergy of today is what we believe will make the global impact of any local operation tomorrow. Looking holistically at the soil health challenge, once the status of the soil health map is established, the results will raise awareness and the potential for the application of newly developed soil amendments in the relevant scientific communities, the general public, farmers, and agricultural representatives. Improved management and recycling of plant residues to improve soil health and reduce nutrient (nitrogen, phosphorus, potassium) discharge to the environment will be our priority, along with empowered interdisciplinary design processes to develop soil amendments that improve root functionality, interact directly or indirectly with plants, and enhance plant byproducts and beneficial microbes in sustainable agriculture. An untapped opportunity lies in the valorization of new environmentally friendly products containing mineral nutrients, organic matter and microbes that can contribute to soil fertility and improvement, taking into account the hierarchy of plant residues focused on prevention measures, followed by reuse and recycling pathways (these residues cannot be used for other higher value purposes). We strongly believe that these smart platform products [smart composting system for targeted crops that enables plant disease control and efficient fertilization, eliminates the negative effects of pest resistance, pollution, toxicity, quality products (functional food for humans and animals) for better human health and soil degradation caused by the use of conventional chemical fertilizers and pesticides] will have a great future.

Keywords: smart biofertilizers; biocontrol; microbiome; sustainable agriculture

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BIODIVERSITY FROM OUR FOOD DISH TO AGRO-FIELDS: THE BIOVALUE PROJECT

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INTRODUCTION

Ecologists have argued that at the farm level, an increase in on-farm species richness and a diversity of overlapping groups of species enhances the level of agricultural biodiversity. This, in turn, increases ecological stability, crop resilience, sustainability and climate protection. Crop biodiversity is an element of agricultural biodiversity, and creates differentiations in soil fauna, weeds, pests, and predators at the farm level and agro-ecosystem. More importantly, crop biodiversity has been reported to increase agricultural productivity through the replenishment of agricultural soils and control of pest infestations, leading to greater farm income security and stability.

STATE OF ART

Crop diversification over the last decades declined due to several driving forces across the food chain (processing technologies, cost impact) as well as consumers' food preferences. Therefore, crops (cereals, legumes, leafy vegetables and fruity vegetables) that can enrich biodiversity significantly are scarcely present in the agri-food value chain and in consumers' food diets since they are no longer economically viable. Decisions regarding the degree of farm-level agrobiodiversity, usually depend on conditions in the relevant food, feedstuff, fuel and fibre markets. In addition to market signals, farmers' agrobiodiversity choices reflect a number of factors aside from market prices, including the social, political, and cultural conditions in which they operate.

EXPECTED RESULTS

The BIOVALUE project aspires to setup a holistic approach to the analysis of the link among the biodiversity, the agro-food value chain, the environment and consumer's preferences and health by developing an innovative, dynamic and modular agent-based simulation tool of the agrifood value chain in order to analyse experimentally the introduction of marginal traditional varieties starting with novel food dish recipes and processed food products and closing the value chain cycle with an extensive breeding program throughout Europe.

DISCUSSION AND CONCLUSIONS

The ultimate goal of BIOVALUE is to enhance and secure biodiversity within farming and the value chain, as sustainable and dynamic value chains combined with fulfilling dietary human needs can determine and maintain biodiversity in European farming systems after the project's completion. The BIOVALUE project goes beyond the state-of-the-art by developing agricultural products that can be certified as ones that promote sustainability through biodiversity. This includes close collaboration with consumers and stakeholders throughout the value chain to ensure the development of healthy, desirable and sustainable products. Most significantly, the BIOVALUE project will develop novel food dishes and processed food products based on the genetically diverse crops, to promote traditional crop varieties and provide comprehensive

solutions on increasing biodiversity at the farm and on consumers' plates. This fork-to-farm, demand-driven, bottom-up approach will provide a clear picture of consumers' preferences and provide further guarantee of the success of the proposed actions.

PROOF-OF-CONCEPT FOR BIOAUGMENTATION OF WHITEWATER FROM WOOD-FREE PAPER MILLS WITH ADAPTED BACTERIA

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Paper industry is one of major polluters of water and an efficient and cost-effective purification technology for the removal of papermaking additives from the process water is still lacking. Therefore, the aim of our study [1] was to develop an effective strategy for treatment of the process water (clear filtrate of the whitewater) using adapted bacterial isolates. We tested a large collection of bacterial isolates individually for their ability to degrade the organic additives used in papermaking, i.e., starch, cellulose, resin acids, alkyl ketene dimers, polyvinyl alcohol, latex, and azo and fluorescent dyes. We developed simple plate biodegradation assays based on iodine complexes and fluorescence and absorbance spectroscopy. By performing 16S rRNA sequencing we identified degraders of recalcitrant azo and fluorescent dyes and best degraders of readily biodegradable additives until genus level. We found that bioaugmentation is more efficient than simply using active sludge treatment, because the microbes apply to Pareto`s law. Namely, among the 318 isolates mostly (80%) below-average degraders of the papermaking additives were found. By using several elimination steps, like cross-activity tests and *co*-culturing, and multivariate statistics we selected a combination of four strains (Xanthomonadales bacterium sp. CST37-CF, *Sphingomonas* sp. BLA14-CF, *Cellulosimicrobium* sp. AKD4-BF and *Aeromonas* sp. RES19-BTP), which cover the entire spectrum of the tested papermaking additives. A proof-of-concept pilot scale study was then performed by immobilizing the artificial bacterial consortium onto porous carriers and introducing them into a 33-liter tubular flow-through reactor with a retention time of <15 h. The combination of the four native strains enabled an 88% reduction in COD of whitewater even after 21 days, which is efficient even in comparison to related work [3].

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GROUP CHAT OR PERSONAL MESSAGE: THE ROLE OF DIFFUSION IN MICROBIAL INTERACTIONS

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Cells communicate with their neighbors by secreting compounds whose movement in the extracellular space depends on diffusion. This results in steep concentration gradients. How do cells communicate with neighbors when their message is quickly diluted? To answer this question we analyzed the effect of diffusion on microbial crossfeeding. In our setup *Lactococcus lactis* secreted glucose, which was sensed by GFP-positive glucose consumers. Cells were grown in agarose-beads that were incubated in water-in-oil emulsions (to prevent diffusion from beads) or in medium (to allow diffusion). We monitored growth inside beads using flow cytometry, and compared the results with a mathematical reaction-diffusion model. Without glucose diffusion from beads, glucose consumers only respond when co-localized with producers. However, when glucose diffused away this specific communication between neighbors rapidly disappears: consumers 15 and 100 µm away from producers showed a similar response, they only sensed the global average. We subsequently developed a protocol for controlled cell aggregation and show that unexpectedly short diffusion-distances are required to allow local and specific communication. These findings might have implications in all domains of life, including intracellular communication, quorum sensing in microorganisms, evolution of microbial consortia and stability of multicellular life.

CA-CASEINATE-ENHANCED REMINERALISATION OF DENTAL APATITE

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The chemical erosion of dental hard tissue, due to acidic drink and food or acidic environment formed microbially from sugar in the oral cavity becomes an increasing problem of modern society. It is estimated that 30-50% of deciduous teeth and 20-45% of permanent teeth are affected by tooth erosion worldwide. Low pH, as well as calcium complexing substances lead to a demineralisation of tooth hard substance by dissolving the main component of dental material, the hydroxyapatite (HAP) $\{Ca_5(PO_4)_3OH\}_2$. Consequences can be increased risk of caries, tooth sensitivity up to tooth loss in extreme cases.[1,2]

One countermeasure to tooth erosion phenomena is the promotion of natural remineralisation processes. Calcium and phosphate ions bound to proteins within the saliva can be incorporated into demineralized tooth structures. This process can be enhanced using Ca-caseinate bio-colloids derived from milk, to increase the bioavailable calcium and phosphate concentrations within the vicinity of the teeth. The $Ca_9(PO_4)_6$ nanoclusters contained in the casein protein structure promote the remineralization of eroded dental structures reversing damage caused by demineralisation events.[3]

Using the technique of white light interferometry, we have tracked the dynamics of demineralisation and remineralisation in vitro with nanometer precision. A roughness analysis of dental dentine using converged Sq parameter [4] maps revealed a heterogeneous reactivity of the tooth surface, i.e. rough areas react stronger, both to de- and remineralisation. Furthermore, first preliminary case studies indicate the potential of a non-intrusive in vivo treatment of tooth erosion diagnosis using Ca-caseinate.

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INTELLECTUAL PROPERTY MANAGEMENT AND FERMENTATION PRODUCT MARKET

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Most important intellectual property form at the public research organization is patent. Patents are granted for any inventions, in all fields of technology, provided that they are new, involve an inventive step and are susceptible of industrial application. A patent shall confer on its owner the following exclusive rights: where the subject matter of a patent is a product, to prevent third parties not having the owner's consent from the acts of: making, using, offering for sale, selling, or importing for these purposes that product. As a rule, patent lasts 20 years from the application date. There are different strategies of geographical patent protection. In accordance with Paris Convention, national or other patent applications can be 'extended' to other countries by filing other national/regional applications. Another option is PCT procedure, where this decision is delayed to 30th or 31st month after priority date.

Intellectual property can be protected also by other forms than patent. When intellectual property is identified, its commercial potential, patentability and cost-benefit of patent should be assessed. It is possible to protect inventions also in the form of secret know-how. In the case a patent application is filed, a scientific article should not be published before the filing date. An example of patent protection is family EP3845633A1. This patent application was first filed in United Kingdom and afterwards at the European Patent Office and Slovenian IP Office.

During the commercialization of the technology, we studied fermentation products market. Most of the market is represented by alcohol products, while sectors cover different areas – food & beverages, agriculture, pharmaceutical, textile & leather, personal care, animal feed and others. Some segments of fermentation market are geographically quite specific. For example, bioethanol is very specific for US, while biogas is very specific Europe. In this study we have also identified several companies, active in fermentation market and segmented them.

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ESTABLISHING NEW VALUE CHAINS IN THE CITIES - EXAMPLE OF THE APPLAUSE PROJECT

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Natural resources on Earth are limited. Today, resources are still too often used only for a short time and then thrown away. Since more than half of the world's population lives in cities, the cities are under large pressing how to meet the needs of the citizens. We need new approaches in city management and more collaboration with different stakeholders, including research institutions. Invasive alien plants are often overlooked by public authorities at local, national and European level. According to the Institute for European Environmental Policy, invasive alien species (both plants and animals) cost EU at least 12,5 billion EUR a year. Within UIA project APPLAUSE, the City of Ljubljana and 10 project partners from different backgrounds managed to develop 65 ways of processing biomass of invasive alien plants into useful products and input materials for industry.

Invasive alien plant species (IAPS) have been recognized for several decades as one of the most important reasons for the decline of biodiversity. They can also cause economic and environmental damage, some are even harmful to human health, as they can cause allergies, skin reactions and inflammation. Like other cities, Ljubljana also faces the problem of IAPS spreading. Since 2014, we are implementing the socially responsible campaign Gloves Up!, which is intended to educate citizens about the harmfulness of IAPS. Citizens' participation was also a basic building block of the UIA APPLAUSE project, in which we have focused on development of new tools for identifying IAPS, education and research of the potential for processing IAPS into various raw materials and products.

Project APPLAUSE enabled gaining new skills within public administration (making better decisions), setting up new participative model – citizens involvement based on 3 levels of engagement, which are adopted to citizen's interests and motivations, increased diversity of educational tools for citizens (DIY catalogue, cookbook, recipe book on pest control, recipe book on dyes, 3D plant prints, videos, festival, collection point, app for plant identification …), development of new green technologies, e.g. pilot enzymatic processing of IAPS fibres instead of chemical and digital tools for IAPS identification and management, processing wood

residues into recyclable 3D composites = zero waste principle. We can make many useful things from leftover materials and cheap, easily accessible natural resources. The use of leftovers in biomass – lignin, dyes and polyhydroxyalkanoates – can be very diverse. Leftovers in biomass can be used to make hybrid colored coatings for glass and wood, natural dyes and as a source of various useful chemicals.

The APPLAUSE project proved that interdisciplinary teams achieve better, innovative solutions. This is especially important in the field of the circular economy, where we are building value chains. The members of the chain are partners who, in most cases, come from different fields of work and use different methods of work in carrying out their tasks. 92 researchers were involved in the APPLAUSE project.

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ABSTRACTS POSTER SESSION

BIOAUGMENTATION IMPROVEMENT USING EDAPHIC MICROBIAL CONSORTIA AND ORGANIC AMENDMENTS

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INTRODUCTION

Petroleum-derived hydrocarbons, which are extensively used in contemporary economies, have severe and unfavourable effects on the environment as a result of oil spills that occur during the processes of extraction, refining, transport, and storage. According to Cai et al. (2016), hydrocarbon pollution in soils, surface waters, and groundwaters poses serious dangers to human and ecosystem health. This pollution is made up of a complex mixture of alkanes, polyaromatic hydrocarbons (PAHs), nitrogen, oxygen, and sulfur-containing chemicals (Gennadiev et al, 2010). In the past, many in situ or ex situ treatments, including biological, chemical, physicochemical, thermal, electric, electromagnetic, and ultrasonic treatment methods, have been used to treat TPHs polluted locations (Ossai et al, 2020). Oil-contaminated soils can be cleaned up by bioremediation techniques, which have been recognized as cost-effective, highly promising and sustainable technologies (Wu et al., 2016). In addition, microbial immobilization on porous supports could improve their survival through the formation of biofilms in the interior spaces of the support. However, there are limitations to using living bacteria, such as the requirement for a constant supply of fresh inocula, aeration, and nutritional supplements (Singh and Walker, 2006).

The aim of this work was to evaluate bioaugmentation methods for bioremediation a polluted soil that contained recalcitrant long-chain hydrocarbons and heavy metals.

MATERIALS AND METHODS

The polluted soil was collected from a machinery park located in Toledo (Spain). More than four years of storage for this soil allowed for some natural attenuation. Air dried soil samples were sieved at 2 mm, and the main properties were: sandy loam texture (11.8% clay, 29.8% silt, 58.3 sand), bulk density 1.51 kg L-1, highest water retention capacity (HWRC) 25.33%, pH (1:5) 7.1, Electrical conductivity (EC 1:5) 0.839 dS m-1, Loss on Ignition (LOI) 3.85 %, Oxidizable Organic Carbon 2.59%, Total N 0.02%, lime content 35.08%. Trace elements (mg Kg-1): As 77.3; Cd 7.8; Cr 14.9; Cu 8.5; Ni 9.9; Pb 339.2; Zn 680.5. Extractable petroleum hydrocarbons (EPHs): 4,051 mg kg-1. As organic amendments, vermicompost (VC) from agroindustrial waste was used as organic amendments at a proportion of 2% (w/w). In addition, two biochars produced from apricot pits pyrolyzed at 450° (BH1) and 650°C (BH2) were used as microbial carriers at a 5% (v/v) ratio; commercial rhamnolipids (RML, Sigma Aldrich) at 1% (w/v) were also tested to increase hydrocarbon mobility.

Different treatments were prepared, either including controls and bioaugmentation (BA) techniques. All the mixtures were prepared using a concrete mixer, in which appropriate amounts of soil, organic amendments, MQ water, modified Bushnell Haas Broth (mBHB) or a suspension of the microbial consortium in mBHB were mixed to reach a final volume corresponding to 40% HWRC. In the case of a second consortia inoculation (day 43th), the soil humidity was increased to a HWRC of 50% in every treatment by adding the calculated amount of BHB solution, with (BA) or without inoculum (CT), or MQ water.

According to Garrido-Sanz et al. (2019), the microbial community was isolated from this contaminated soil. A small amount of consortia was suspended in a minimum medium with diesel as the only carbon source (10 mL L-1) and incubated overnight at 150 rpms and 28 °C. The culture was centrifuged, resuspended, and appropriately scaled. After centrifuging all of the cultures for 30 minutes at 3,800 g and 8 °C, they were all washed to eliminate any remaining of diesel.

Soil microcosms (200 g) were incubated at 22 °C in the dark for 2, 15, 30, 45, 60 and 90 days. At day 43, a second inoculation was performed. Aeration of the soil mixture and a humidity control were performed twice a week. At the end of each sampling point, samples were divided to perform different types of analysis.

Analysis of EPHs and PAHs were performed according to Pindado-Jiménez et al. (2014), and the physicochemical properties determined according to standard analytical methods. One-way ANOVA and LSD post-hoc test were applied to seek for differences between treatments.

RESULTS AND DISCUSSION

This polluted soil was stored for a period higher than two years and most of EPHs had disappeared by natural attenuation. After 90 days of incubation, the EPHs content in this polluted soil accounted for 3,806 ± 225 mg kg-1 DM, most of them aliphatic hydrocarbons (LAHs; 83%), with a predominance of high molecular weight compounds (71.5 % in the molecular range of C22-C35). PAHs accounted for the rest (17 %), mainly in the range EC22-EC35 and >EC35.

The degradation of the different fractions of hydrocarbons after VC treatments clearly displayed a statistically significant decrease in the concentration of these heavy fractions of hydrocarbons. Also, chromatograms displayed a strong degradation of low molecular weight hydrocarbons, mainly linear and branched hydrocarbons. However, there was a persistence of the more recalcitrant compounds such as high molecular weight PAHs and hopanes.

Results obtained after biochar incubation showed the high recalcitrancy of these EPHs to microbial degradation, in which only the introduction of biochar, mainly with BH2, slightly reduced (around 10 %) the total content of EPHs. On the contrary, bioaugmentation treatments displayed a higher hydrocarbon degradation capacity by the action of the added microbial consortium alone (BA4CT, 23 %) or after immobilization onto biochars (BABH1 and 2) or with the addition of rhamnolipids as biosurfactants (BARML), all of them with yields close to 30 % of initial EPHs' concentration.

The characteristics of the soil, with a scarce presence of soluble or available nutrients such as N and P, supposed an important constrain to soil biological activity. The addition of BHB medium in the suspended consortium supposed an important increase in extractable nitrogen or available phosphate, exceeding the nutrient demand of the added consortium.

CONCLUSIONS

In this study, the degradation of EPHs was not taken into consideration in their heaviest and more recalcitrant fractions, probably strongly linked to the mineral matrix of the soil, which is an important element to consider in future strategies. The introduction of a microbial consortium, nutrients and organic amendments increased the biological activity in the soil, but not the bioaccessibility to the targeted contaminants. The use of new materials as carriers for microorganisms, which may increase the bioavailability of the more resistant substances, is the focus of current research, to improve the bioremediation techniques.

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STUDY OF THE TOXICITY AND THE ANTIMICROBIAL ACTIVITY OF DIFFERENT FORMS OF ZNO NANOPARTICLES: ZNO NANOPARTICLES LINKED TO GRAPHENE, PRISTINE ZNO NANOPARTICLES AND ZNO NANOPARTICLES DOPED WITH MN

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Zinc oxide nanoparticles (ZnO NPs) are widely used in many industrial sectors (rubber, paint and pigment, ceramic, photocatalysis, electronics and electrotechnology)1–3, personal care products (medical ointments, sunscreens and cosmetics) 4–6 and agriculture due to their special physico-chemical properties. Moreover, in the last two decades, these nanoparticles have attracted interest in different biomedical applications such as anticancer therapy, drug delivery, antibacterial, diabetes and anti-inflammation treatment, wound healing and bioimaging 7–10, since in comparison with other metal nanoparticles, ZnO NPs are inexpensive and relative less toxic 11.

In the present study, the toxicity of different zinc oxide nanomaterials (G-ZnO, ZnO and ZnO:Mn) was assessed taking in account their application, employing in vitro cellular models representative of the main exposure routes. In addition, their antimicrobial properties were studied in different relevant pathogenic bacteria. G-ZnO NM presents catalytic and photocatalytic activity and excellent properties such as supercapacitor. Considering that inhalation is the major exposure route to G-ZnO in the industrial production 12, the possible toxic effects on the respiratory system were determined using the human alveolar carcinoma epithelial cell line A549. On the other hand, the ZnO and ZnO:Mn NPs are applied in sunscreens and cosmetics, hence, the first route of the exposure to these NMs is the skin. Regarding this, their irritant potential was evaluated by reconstructed 3D human epidermal model EpiDerm™. The obtained results showed that G-ZnO nanomaterial causes a reduction on cell viability and the induction of the reactive oxygen species (ROS) production. In the case of ZnO and ZnO:Mn NPs, any of them caused a reduction of the skin tissue viability in the studied conditions. In addition, the antimicrobial activity of G-ZnO, ZnO and ZnO:Mn was analysed against Gram positive (methicillin-resistant S. aureus and vancomycin-resistant E. faecium) and Gram negative bacteria (A. baumannii and P. aeruginosa) bacteria strains. None of the studied NMs presented activity against the selected Gram positive and Gram negative bacteria in the established conditions (MIC ≥ 256 μg mL-1).

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INVESTIGATING THE FUNCTION, PERSISTENCE, AND BIOSAFETY OF CONSTRUCTED MICROBIOMES FOR IMPROVED BIOREMEDIATION OF PETROLEUM-IMPACTED SOIL

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INTRODUCTION

Soil is one of the most important non-renewable resources available to mankind. Yet over the last 100 years industrial and agricultural activities have resulted in serious degradation and pollution of soils across the globe. The extraction, processing, storage, and use of crude oil and its derivatives have left a legacy of contaminated sites and pose a grave threat to the health and well-being of human beings, animals, and plants. In Europe, there are estimated to be around 2.5 million contaminated sites, of which about 14% are considered to be heavily contaminated (Agency, 2014), If current trends continue, the number of sites needing remediation is expected to increase by 50% by 2025 (Boots, 2017). Hence, these sites immediately need to clean up (remediate). Bioremediation is the use of microorganisms that have the ability to metabolize petroleum compounds, decreasing their concentration and associated health risks. IT Carlow has developed a patented process (Ecopiling) for cleaning up contaminated soil and has developed a mixture of oil-degrading bacteria (the consortium). However, the effectiveness of the application of bacteria to contaminated soil to speed up removal rates is often questioned, as in many cases the survival, function and persistence of these introduced microbes are not determined. Therefore greater understanding is needed of the survival, persistence, biosafety, and effectiveness of inoculated bacteria in the remediation process. This project will analyze consortia of oil-degrading bacteria for their safe use in the environment (using standard ecotoxicity assays (Daphnotox/Phytox), will use -omics technology to investigate the fate of the consortia in the sol and its effects on the soil microbiome, and will determine the key genes that are involved in the oil degradation process.

STATE OF THE ART

Developed a multiprocess bioremediation system known as Ecopiling. The Ecopile process involves biostimulation of indigenous hydrocarbon degraders, bio-augmentation through inoculation with known TPH degrading consortia, and phytoremediation, through the effect of root growth and penetration throughout the soil and the resulting stimulation of microbial activity in the rhizosphere (Germaine et al., 2015). Although the ecopiling process was a success, we have little understanding of the microbial processes that lead to this success. This research focuses on understanding the role that the inoculated consortia had in the remediation process. This research aims to select high-effect oil degradation bacteria and build up a high-effect oil degradation bacterial consortia, assessing the Biosafety /eco-toxicity and validating the degradation ability in trial plots experiment. Finally, utilize the soil DNA data to examine the correlations of the diversity and abundance of key degradation genes with the microbial microbiome, and carry on transcriptomic analysis of functional biodegradation genes.

RESULTS

In the bacteria microbiomes, twenty classes accounted for 87.5% of the bacterial community. The remaining 12.5% was made up of 160 different classes. Gammaproteobacteria made up 34.6% of the reads. The Alphaproteobacteria was the second largest group (an average of 19.9%). The other group represents the total relative abundance of the 160 types remaining phyla besides the top ten (12.44% average).

In the fungal microbiomes, twenty genera accounted for 56.3% of the fungal community. The remaining 43.7% was made up of 356 different genera. The most dominant fungal genus Mortierella made up 14.31% of the reads. Doratomyces was the second largest group (an average of 5.51%). The other group represents the total relative abundance of the 356 types remaining phyla besides the top ten (12.44% average).

In the Nematoda microbiomes, nine genera accounted for 99.99% of the Nematoda community. The remaining 0.01% was made up of 2 different genera. Rhabditida made up 67.68% of the reads. The other dominant class, determined using average group relative abundances, accounting for >1% of the soil microbiome include Diplogasterida (17.54% on average), Araeolaimida (6.40% on average), Tylenchida (4.40% on average) and Monhysterida (2.70% on average). Triplonchida (0.65% average) and Aphelenchoides (0.42% average) account for just below 1% in average. Dorylaimida and Oxyurida have less than 0.1% on average.

High oil degradation bacteria were selected based on their growth ability in a diesel environment. The degradation ability analysis of selected bacteria was then carried on, GC analysis shows individual bacterial B4 has the lowest area which means it has the highest Diesel degradation ability. The degradation ability of all 5 selected bacteria was: B4>M9>D11>E9>D17.

DISCUSSION

Regarding the bacterial observed relative abundance, for most of the Ecopiles the distribution at the December 2019 timepoint was similar to the distribution of the starting of the process, with Gammaproteobacteria representing almost 50% of the abundance, Alphaproteobacteria with a 20%, and Actinobacteria, Bacteroidia and Bacilli with values near 10%.

Regarding the Nematoda observed relative abundance, most dominated genera have an increase and then decrease trend, the most dominated genus Rhabditida representing 65% in July 2019, 77% in February 2020, and 54% in November 2020.

CONCLUSIONS

The phylum Proteobacteria and (Gammaproteobacteria at class level) for bacterial, and genus Rhabditida for Nematoda, whose relative abundance varied with the TPHs' levels and was associated with the samples of the first timepoint, which were more contaminated, could have played a major role in the degradation of these pollutants.

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INSIGHT INTO GENOMIC DNA OF THE SELECTED STRAINS FROM ORAL CAVITY WITH ANTIMICROBIAL ACTIVITY AGAINST PARODONTAL PATHOGEN USING THE NANOPORE TECHNOLOGY

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INTRODUCTION

Periodontal disease represents one of the biggest threats to dental health. It is an infection-driven inflammatory disease affecting the tooth-supporting tissues, which in the late phase can lead to bone destruction and tooth loss. The inflammation is triggered by different factors where the periodontal pathogens such as Aggregatibacter actinomycetemcomitans give the highest impact on the disease outcome. In the oral cavity of healthy individuals we found bacteria that can have a protective role from the invasion of pathogens by ecological interference through competition and antagonism against A. actinomycetemcomitans. However, the mechanistic information of this ecological interference resembled unknown and therefore our goal was to determine this potential through the analysis of genomes. Here, six selected strains 27.3.Z, 4.1.Z, 25.1.S, 28.1.J-2, and strain 27.3.S as well as strain 26.3.J, belonging to Bacillus, Stenotrophomonas and Staphylococcus genera, respectively.

METHODS

We extracted bacterial genomic DNA with our newly developed protocol which is based on combined detergent and enzymatic extraction with further gTUBE fragmentation to gain more evenly distributed sizes of fragmented DNA. After sample preparation we sequenced genomic DNA using a nanopore MinION device. The further bioinformatics analysis included basecalling using Guppy basecaller, assembly with Canu assembler, 16S rRNA gene identification using Barrnap and for nearest neighbour identification we used Blast and for identification of secondary metabolites we used antiSMASH.

RESULTS

The genome size of Bacillus strains ranged between 4,16 and 4,2 Mb. Based on the 16S rRNA gene comparison the nearest neighbours belong to Bacillus velezensis species. For Stenotrophomonas strain the genome size is 4,54 Mb and based on the 16S rRNA gene comparison the nearest neighbours belong to Stenotrophomonas maltophilia species. The genome size of Staphylococcus strain is 2,73 Mb and based on the 16S rRNA gene comparison the nearest neighbours belong to Staphylococcus succinus strains. Using the antiSMASH software we identified a variety of secondary metabolites at different genome regions in selected strains that belong to different gene cluster types. The common gene clusters that might have ecological interference impact between our strains and odontopathogens were for surfactin, bacilysin, bacillaene, bacillibactin, amylocyclicin, fengycin, macrolactin H, paenibacterin in our Bacillus strains. Additionally identified gene clusters belonged to phosphonates, Type III PKS and terpenes. In the Stenotrophomonas strain we identified RiPP like, NRPS and arylpolyene gene clusters. In the Staphylococcus strain we identified NRPS and NRPS-like, type III PKS, siderophore, terpene and cyclic-lactone-autoinducer gene clusters.

DISCUSSION

Based on research articles, related to the potential of secondary metabolites that show antimicrobial effects against different gram negative bacteria, our prediction is that selected strains secrete more different types of secondary metabolites that may contribute to prevention of periodontal disease in healthy individuals. The identified metabolites belong to terpene, type III PKS, PKS-like, NRPS and beta lactone gene clusters.

CONCLUSION

We identified genetic determinants for the production of secondary metabolites that may affect the periodontal pathogen A. actinomycetemcomitans, but the further analysis of each of the identified secondary metabolites and their effect on the pathogen is needed to define the mechanisms that take action against the selected pathogen.

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METAGENOMIC CHARACTERISATION OF AN ENRICHED MICROBIAL COMMUNITY CARRYING OUT SIMULTANEOUS BIOELECTROCHEMICAL REMOVAL OF AZO DYE AND CHROMIUM FROM DYEING PROCESS EFFLUENT

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INTRODUCTION

Azo dyes are the most frequently used class of organic dyes in the paper, textile, pharmaceutical, cosmetics and food industries, accounting for 50% of global dye production. They are characterised by N≡N bond(s) between two structurally diverse alkyl or aryl groups. Although azo dyes are stable, resistant to light and microbial degradation, many azo dyes and their breakdown products are considered harmful to human and animal health and the environment. Dyeing process effluents (DPE) are the primary source of azo dye pollution; however, conventional wastewater treatment technologies are ineffective for the removal of the azo dyes.

STATE OF THE ART

Bioelectrochemical systems, specifically Microbial Electrolysis Cells (MEC), are a promising technology for the treatment of DPE. In MECs, voltage is applied to the system by an external power source, and the resulting current facilitates the reduction of the target pollutants (such as dyes and metals REFERENCE) at the cathode. The reduction process is partially facilitated by microbial activity at the cathode. In this work, DPE containing several chromium-complexed azo dyes was treated in the cathodic chamber of a two-chamber liquid MEC with an abiotic anode.

RESULTS

The cathodic chamber (125mL) of the two-chamber MEC was inoculated with microbial samples obtained from river sediments and soil polluted with heavy metals. The DPE was diluted with minimal medium (1:3) and a voltage of 2V was applied. Dye decolourisation was measured by absorbance at 574nm, and chromium concentration was measured using Microwave-Plasma Atomic Emission Spectroscopy (MP-AES). Decolourisation efficiency (DE) and chromium removal efficiency (CRE) up to 90% were observed following enrichment of the cathodic community and substantial DE and CRE were demonstrated throughout long-term operation of the reactor. DNA was extracted after long-term enrichment of the cathodic biofilm. The metagenome of was determined by shotgun sequencing and in-silico assembly of Metagenome Assembled Genomes (MAGs). The most prevalent MAGs were taxonomically assigned to Comamonas testosteroni (17.9%), Pseudomonas stutzeri (10.9%), Cupriavidus campinensis (8.3%), Phenylobacterium sp. (5.7%), Xanthobacteraceae 62-47 (5.1%), Bosea sp. (4.7%), Parvibaculum sp. (4.7%), Brevundimonas sp. (3.8%), Sphingomonas sp. (3.6%), Stenotrophomonas maltophilia (3.1%) and Afipia birgiae (2.6%). MAGs were searched for putative azoreductases and other reductases potentially involved in azo dye and chromate reduction, chromate reductases, chromium

resistance genes, as well as hydrogenases and multiheme cytochromes potentially involved in cathodic electron transfer processes. Several putative azoreductases were identified, while genes for chromium resistance was detected in nearly all MAGs. A decaheme cytochrome was found in the MAG of Strentrophomonas maltophilia, potentially involved in direct electron transfer.

CONCLUSIONS

The results presented here demonstrate the successful enrichment of a chromium-resistant microbial community capable of effectively and simultaneously reducing both azo dyes and chromium in MECs, thereby reducing the toxicity of DPE. The results of the bioinformatics analysis will be used to construct a synthetic community free of potential pathogens with the ability to remediate contaminated DPE using MEC technology.

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METAL(LIOD)S REMOVAL FROM POLLUTED GROUNDWATER WITH BIOELECTROCHEMICAL SYSTEM AND PHYTOREMEDIATION

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INTRODUCTION

The polluted water containing high levels of targeted metals can be treated with BES (Molognoni et al. 2021). The metals are removed due to precipitation and deposition in the cathodic chamber in a multistage process; however, the exhausted anolyte and treated catholyte are produced. Further, the physical and chemical parameter (high salinity and extreme pH) of the treated catholyte is another challenge, as it presents a high level of ecotoxicity. High levels of metals and metalloids in groundwater can be treated using plants; nevertheless, the acute stress due to exposure results in the generation of less biomass, and the treatment time needed results in limiting the benefit of the phytoremediation system (Khan et al. 2022). The individual limitation of both remediation systems (ecotoxicity for BES and time for phytoremediation treatment) can be removed by coupling these together in series. In this way, polluted water first flows into the BES, where the metals are removed/recovered in the catholyte chambers, operating at the different applied potential to remove metal from water, in batches. The resulting treated groundwater and exhausted anolyte are being fed to subsurface flow constructed wetland to which utilize the anions and further reduce cation in treated water. collected from HRT experiment at 15 and 30 days internal. All the materials used were previously washed with diluted HNO3 to avoid possible traces of contamination.

STATE OF THE ART

Following scheme of treatment was used for the experiment.

Initially, a characterization of the real groundwater samples (Sites _008 and _009) shipped from TAUW and treated groundwater mixed with polluted water were performed upon their arrival, aimed to confirm and double check the concentrations of the potentially toxic metal(loid)s on the water samples under study. Target metals, together with other elements either major and trace, were determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry with measurement in radial mode, GENESIS, Spectro), and ICP-MS/MS (Inductively Coupled Plasma Mass Spectrometry triple quadruple, 8900, Agilent), respectively.

RESULTS

The Sequential strategy MFC + MEC, was carried out doubling the treatment time per volume of water as two batches (one in MFC first, and then one in MEC mode) were done to treat the same groundwater. In that case the total metallic pollutants removal was 38% after MFC treatment and 91% at the end, i.e. after MFC+MEC. This is 24% more than in Pilot-MEC-V experiments. In

the MFC step, Cu and Fe were almost completely removed accounting for a removal efficiency higher than 99,9% in both cases. Also As was significantly removed (90%). In the second step (MEC treatment) Al, Ni, Zn and Cd were successfully removed achieving a removal efficiency of 92%, 71%, 76% and 87%, respectively. Regarding the pH, in the 1st step it raised from 2.66 to 4.06 and in the second step it reached 4.65 in the final solution. Both were lower than metal hydroxides precipitation pH (for most of the studied metals).

The physiological response of aquatic plant cultivated clean water, BES treated water and polluted water showed notable physiological differences. The canopy cover of P. australis and S. holoschoenus was much broader than T. angustifolia during the experiment. It indicates that the plants have variable response towards the provided condition. At the time of harvest, the status of biomass production also showed the same results. Clearly, the plants of P. angustifolia and S. holoschoenus showed no change in physiology between the comparison of plant cultivated in control conditions with plant cultivated on BES and PW. The impact of HRT was found on T. angustifolia, which showed less impacted plant physiological response at HRT of 30 days, than in HRT of 15 days. At the time of harvest, the status of biomass production also showed the same results. Clearly, the plants of P. angustifolia and S. holoschoenus showed no change in physiology between the comparison of plant cultivated in control conditions with plant cultivated on BES and PW. The impact of HRT was found on T. angustifolia, which showed less impacted plant physiological response at HRT of 30 days, than in HRT of 15 days. The water after BES treatment followed by phytoremedation, with HRT of 15 and 30 days showed limited to no metal(liod)s contents, and the metal(liod)s content was within the permissible limits of groundwater Sanitation Standards of Flanders (Coetsiers et al. 2009). Further, despite this was considerable reduction in the metal(loid)s content, with phytoremediation the level of As, Cd, Fe, and Pb remained within the permissible limit of groundwater Sanitation Standards of Flanders.

CONCLUSIONS

- The strategy MFC + MEC to sequentially recover copper in a first step and remove the rest of the pollutants in a second step is feasible if the handicap of the electroactivity loss is solved. Different strategies such as to add a third buffer chamber and a second separator in the BES reactor are being considered.
- The choice plant for integration and HRT to the management of remining metal(liod)s and byproduct of BES is essential, which was also evident in present integration trials. Only P. australis and S. holoschoenus showed no to limited impacted plant morpho-physiology.

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TOXICOLOGICAL ANALYSIS OF VIOLOGEN DERIVATIVES FOR APPLICATION IN REDOX FLOW BATTERIES

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The current energy transition to renewable energy resources makes the development of energy storage systems a priority for the international community. Among the different systems studied, redox flow batteries are considered as promising alternatives. However, those currently commercially available relies in vanadium species, which is a great handicap since this element is considered a critical raw material by the USA and the EU. In this context, organic redox active species such as methyl viologen have emerged as an excellent alternative due to its outstanding electrochemical properties but the toxicity associated to this molecule is one of its major drawbacks.

The aim of the present work is to evaluate applying in vitro assays the toxicity of six molecules viologen-derivates with the aim of elucidating whether toxicity is an intrinsic of this family or this key parameter can be tuned. Thus, different model organisms representative of human and environmental exposure were used. The ability of these compounds to affect viability and induce oxidative stress in the lung cell line A549 and in the yeast Saccharomyces cerevisiae was studied. Furthermore, the effect of these compounds on the bioluminescence of the Gram-negative bacterium Vibrio fischeri was analysed. In general terms, from a toxicological perspective, the obtained results indicate that the modification of viologen with functional groups (SO_3, SO_3-monosubstituted and NC_3H_9) is a better alternative than the addition of -CH groups to the side chains to reduce the toxicity of this molecule.

PHYTOREMEDIATION AND ANALYSIS OF SOIL CONTAMINATED WITH PETROLEUM HYDROCARBONS

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INTRODUCTION

Contamination of soil is an issue of global concern that continues to grow, becoming an increasing burden on both the environment and society. There are many different pollutants that contribute to this; those of greatest concern include petroleum hydrocarbons (PHC), and heavy metals (Devatha et al., 2019). The petrochemical industry continues to grow due to a combination of population growth and demand for petroleum derived products. This growth contributes to increasing petroleum hydrocarbon pollution, which occurs during production, transport, usage or disposal of the oil or waste products. Once released into the environment, PHCs may form slicks on the surface of water bodies or accumulate in soils and sediments (Baoune et al., 2019;). The physical and chemical interactions of soil and petroleum hydrocarbons make this type of soil pollution particularly harmful to the environment and challenging to resolve.

STATE OF THE ART

The landscape of environmental remediation is going through a necessary period of transition. Thermal treatment and chemical oxidation are two established methods that efficiently remove contaminant from soil but, in the process, alter or destroy the soil itself. Phytoremediation is an alternative candidate to these established techniques and can most simply be defined as the use of plants to degrade or remove environmental contaminants. It is often paired with bioremediation (using bacteria) and is an ever-improving method of removing or degrading PHCs from polluted soil. (Quintella et al., 2019). Phytoremediation is considered an attractive alternative because it is cost-effective, ecologically beneficial, in situ, passive and is more aesthetically pleasing. It utilises the natural activities of plants and their associated microorganisms to degrade contaminants in an effected ecosystem. Rhizodegradation is said to be the most suitable for PHC contaminated soils. This is a direct degradation technique, where the plants and microbes work together to directly metabolise the PHCs. Other factors, such as addition of soil amendments and use of endophytic microorganisms can also improve the efficacy of this approach (Hussain et al., 2018.

RESULTS

Tukey Pairwise Comparisons					
Grouping Information Using the Tukey Method and 95% Confidence					
Treatment	**N**	**Mean TPHs**	**Grouping**		
Initial level	3	7418	A		
Chicory	5	5737	A	B	
SF Mix	5	5049	A	B	C
Control	5	4450	A	B	C
Abergreen	5	3268		B	C
Abergain	5	3238		B	C

Mustard	5	2353			C
Means that do not share a letter are significantly different.					

Table 1 is a Tukey pairwise comparison of all treatments and concentration of TPHs to determine if there is a significant difference between treatments, controls and initial level. Analysis done in Minitab V20.4 and collated in Excel.

DISCUSSION

The aim of this trial was to determine if phytoremediation treatments would have a positive effect on the degradation of petroleum hydrocarbons in soil. The study found that three out of the six plant treatments degraded the initial total petroleum hydrocarbon level by a statistically significant margin ($P<0.05$). These were Mustard (Sinapis alba) Abergain and Abergreen (both Lolium perenne). The control, Chicory (Chicorium intybus) and Sugar Factory mix (Lolium perenne and trifolium repens) did not reduce TPHs by a statistically significant amount compared to the initial level ($P<0.05$). A TPH certified reference material was used as a standard to create calibration curves capable of assessing PHC degradation increments based on carbon chain length. Fractions were separated in increments of two carbons starting at c8-c10 up to c28-c40 for a total of 16 increments. Except for the c8-c10 range, Mustard had the lowest mean concentration for every fraction of hydrocarbons. Of the total 16 fractions that were analysed, Mustard reduced 12/16 fractions by a statistically significant margin from the initial level. Mustard reduced 10/16 fractions better than the control by a statistically significant margin and significantly outperformed all other treatments in 5/16 fractions. No treatment achieved better degradation than Mustard in any fraction by a statistically significant amount.

CONCLUSIONS

There was no correlation found between total, root or stem and leaf plant biomass and TPH degradation, but Treatment and TPH degradation correlated strongly. As the plant biomass does not appear to be a factor in success of phytoremediation in this instance, other elements must be investigated. The most likely candidates are microbiome structure and abundance. Soil was sampled at multiple stages during this pot trial to track the progress of the microbiome over the course of the trial. Plants select and influence the microbial community in and around their rhizosphere through the release of root exudates. These may attract specific microbes to the root zone or promote processes like co-metabolism to boost the metabolic capability of the microbial community. If plants like Mustard are shown consistently to boost bioremediation through plant-microbe interactions, it may shift focus away from the traditional approach of using plants with fibrous roots such as Lolium perenne to novel or combined systems with a primary focus on bolstering the microbial communities' metabolic capacity.

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PHYSIOLOGICAL AND TRANSCRIPTOME PROFILING OF CHLORELLA SOROKINIANA: AN AZO DYE WASTEWATER DECOLORIZATION STUDY

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INTRODUCTION

Over decades, synthetic dyes became increasingly dominated by azo dyes – a group characterized by one or more diazenyl functional groups (R N=N R´) which represent more than 60 % of the dyes produced worldwide [1]. As a consequence of large-scale production and a low degree of degradation, wastewater containing azo dyes can accumulate in the soil, sediments, and water systems [2], potentially resulting in environmental toxicity. Given the demand for more efficient and environmental solutions for wastewater treatment, biological techniques help to address these issues. Microalgae-based systems are one of these bioremediation strategies capable of processing wastewater without aeration [3] coupled with potentially valuable biomass production (biofuels, biofertilizers, biochar) [4]. This study aims to verify the potential utilization of microalgae for industrial wastewater treatment, emphasizing the regulation of molecular mechanisms to cope with such a harsh environment.

STATE OF THE ART

The current state of the art in microalgal bioremediation is focused on finding the tolerant strains, optimizing physicochemical conditions, or applying different adsorbents or supplements with microalgae to increase the removal efficiency of pollutants with emphasis on the underlying mechanism of their bioremediation (biosorption, bioaccumulation or biodegradation). Recently, circular bioeconomy, meaning removing pollutants with simultaneously producing valuable compounds is also discussed. Currently, research is also focused on multi-omics approaches revealing comprehensive mechanisms underlying the tolerance of microalgae to complicated wastewater conditions, bioremediation strategies, and enzymatic dynamics of microalgae, promoting the development of sustainable bioremediation technology.

RESULTS AND DISCUSSION

Based on the preliminary microalgal screening test, Chlorella sorokiniana showed the highest decolorization efficiency indicating strain tolerance against nutrient imbalance and the presence of toxic compounds in wastewater compared to other tested microalgae [5]. Furthermore, tolerance of strain used in this study was evidenced using multiple approaches (growth and chlorophyll content assays, scanning electron microscopy (SEM), and antioxidant level measurements) with no adverse effects detected. Subsequently, optimization of the experimental design resulted in 70 % decolorization. In previous studies, higher decolorization efficiency of Chlorella was observed in response either to Congo red (83 %) [6], Direct-red 31 (96 %) [7], or synthetic wastewater containing azo dye (99 %) [8] compared to real wastewater (42 – 50 %) [9] indicating that high salinity, high chromaticity, and the complex composition of dyes in industrial wastewater might hinder decolorization processes [10]. Further, Raman microspectroscopy was employed for the quantification of azo dye wastewater-specific compounds accumulated by microalgae biomass. Finally, RNA-seq revealed the transcriptome profile of C. sorokiniana exposed to azo dye wastewater for 72 hours. In total, 1249 genes were

annotated, which included 714 upregulated genes and 701 downregulated genes compared to the control. In terms of the decolorization and detoxification process microalgae significantly upregulated several transporter genes and oxidoreductases-, and glycosyltransferases-encoding genes to sequestrate and detoxify azo dye wastewater effectively.

CONCLUSIONS

C. sorokiniana showed potential as an efficient decolorizing (70 %) and tolerant strain since no adverse effects (specifically growth, morphology, and oxidative stress) were detected. In addition, a proof-of-principle experiment was performed using Raman microspectroscopy to quantify azo dye wastewater bioaccumulation in Chlorella. Moreover, the effluent evaluation indicated lower toxicity than before bioremediation. In terms of trancriptomic profiling of microalgae, changes in carbon metabolism, including photosynthesis, the TCA cycle, or glycolysis, were critical for energy support sufficient for the growth of the treated cells. Specifically, the identified transcripts pointed to the reversal of alkylation damage in the DNA and excision repair pathways. RNAseq data also revealed that microalgae significantly upregulated several transporter genes, oxidoreductases-, and glycosyltransferases-encoding genes to tolerate and detoxify azo dye wastewater effectively.

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AN ELECTROSTATIC APPROACH FOR CONSTRUCTING BIOREMEDIATION EFFICIENT CONSORTIA BY RANDOM COMBINATION OF UNCULTIVATED MICROBIAL CELLS

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Oil pollution is a prevailing environmental issue threatening marine and terrestrial ecosystems. Hydrocarbons found in oil are highly toxic to animals, plant and microbial communities and natural attenuation is slow and often inefficient resulting in accumulation of recalcitrant fraction. Currently, microbial bioremediation techniques are used in combination with various chemical and mechanical methods, depending on the severity of oil spills and concentrations of the pollutants in the environment. The complexity and diversity of the substrates in the pollutant led to the use of bacterial consortium where multiple species with different metabolisms are involved in the degradation process. Degradation of complex substrates by microbes is often a process involving metabolic pathways, where a product of one microbial species is a substrate for another one. Furthermore, oxidation of the pollutant can require additional electron shuttling molecules or mediators and various auxiliary enzymes, not directly involved in the degradation, but can be a key component in the efficient bioremediation. While microbial communities are therefore composed of bacteria with a specific role, not all members are beneficial to the community or to the degradation of the pollutant. Communities can contain "cheating" bacteria that are feeding on the products other members of the community produce and are not contributing to the overall degradation. Artificial communities of bacteria can be built by selecting the most efficient strains and by constructing microbial consortiums, but the issue that still prevails is the distance between bacterial cells, disabling effective compound exchange, uncontrolled growth of bacteria and no spatial distribution of the cells. To build an artificial bacterial community most optimal for degradation of oil, we developed methods based on a combined approach of colloid biology and microfluidics to out select the smallest and the most efficient oil degrading consortium. Aggregation of bacterial cells in this process is crucial, since closing the distance gap between the cells enables effective compound transfer, which is essential for co-metabolic pathways to take place between different bacterial strains.

The method is based on random aggregation of the suspension of cells from isolated oil degrading consortia and by immobilisation in micro-sized alginate beads that are acting as microcontainers. Using microfluidic approach, a large amount of beads entrapping aggregated consortia can be produced in a short amount of time, where members of consortia further grow and multiply using specific carbon source. To assess the oil degrading ability of each bacterial cell combination in the bead, a redox dye 2,6-dichlorophenolindophenol (DCPIP) is added to initial alginate suspension, along with the nutrients required for bacterial growth. Such an approach of screening for the smallest and most optimal bacterial community for oil degradation can out-perform classical screening techniques through the use of less space, getting more combinations and obtaining spatially oriented combinations of strains. In order to monitor developing consortia we used fluorescent microscopy to check the viability and growth, while flow cytometry was used for bead characterization. Beads up to 20 µm diameter size were produced and consortia from 14 beads with the highest DCPIP signal were further on isolated.

Degradation ability of isolated communities was checked by measuring the reduction of DCPIP signal spectrophotometrically and obtained consortia were further subjected to genomic analysis.

This new approach enables rapid and efficient selection of the most optimal bacterial consortia not only for bioremediation of environments contaminated with oil but also for biotechnological processes where particular multispecies processes are demanded. By isolating different randomly generated bacterial communities and allowing them to grow in alginate beads we were able to acquire combinations with different efficiency of degradation and ultimately select the most effective one. The developed method provides an alternative approach to bacterial isolation and screening based on cell aggregation and rapid bead encapsulation, that are trying to address the current issues with bioremediation.

LAB-ON-A-CHIP FOR THE EASY AND VISUAL DETECTION OF SARS-COV-2 IN SALIVA BASED ON SENSORY POLYMERS

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The initial stages of the pandemic caused by SARS-CoV-2 showed that early detection of the virus in a simple way is the best tool until the development of vaccines. Many different tests are invasive or need the patient to cough up or even drag a sample of mucus from the throat area. Besides, the manufacturing time has proven insufficient in pandemic conditions since they were out of stock in many countries. Here we show a new method of manufacturing virus sensors and a proof of concept with SARS-CoV-2. We found that fluorogenic peptide substrate of the main protease of the virus (Mpro) can be covalently immobilized in a polymer, with which a cellulose-based material can be coated, and the results showed that the higher the hydrophilicity of the copolymer, the higher the performance of the sensory labels. These sensory labels fluoresce with a single saliva sample of a positive COVID-19 patient. The results matched with that of the antigen tests in 22 of 26 studied cases (85% success rate).In addition, the proposed methodology can be applied to detecting saliva or other body fluids and expired air for virus and bacterial infections.

INFLUENCE OF MICROBIAL AND ORGANIC FERTILIZERS ON BACTERIAL COMMUNITIES COMPOSITION DURING KEY GROWTH PHENOPHASES OF MAIZE

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INTRODUCTION

Maize is among the three world's most important and widely grown cereals (Seyi-Amole & Onilude, 2021). The excessive and long-term application of agrochemicals for providing maize with essential nutrients, required for the development of all growth phenophases and for yield enhancement, leaves adverse consequences for human health and the environment (Khaliq et al., 2004). Although their use is inevitable to meet the increasing demand of the growing human population for a healthy food supply, organic fertilizers and biofertilizers (microbial fertilizers) are becoming recognized as effective, economically feasible, and environmentally sound alternatives for sustainable agriculture (Lawal & Babalola, 2014; Hui et al., 2017; Mahanty et al., 2017). The main objective of this study was to evaluate the influence of the microbial inoculant Phytobiotic (PHY), containing a consortium of Bacillus subtilis sp. subtilis and Microbacterium sp., on native maize microbiome during key growth phenophases (seedling, flowering, and harvesting) under field conditions, as well as to compare whether differences in efficacy between PHY, poultry manure (PM) and their combination (PHY_PM) exist, based on yield parameters.

STATE OF THE ART

Seeds, roots, and soil samples were taken for metabarcoding analysis during four growth phenophases (I-IV). Samples of uninoculated seeds and soil, poultry manure, and seeds inoculated with PHY were primarily taken before sowing (phenophase I). Further, during the growing season [phenophases II (seedling), III (flowering), and IV (harvesting)] the effect of PHY, PM, and PHY_PM on maize seeds, roots, and soil microbiome was evaluated in relation to concurrently sampled negative controls. A total DNA from the collected samples was isolated, amplified with primers 515F/ 806R targeting the V4 region of the 16S rRNA, and subjected to next-generation sequencing (NGS). The obtained sequence data were bioinformatically processed and used for the evaluation of alpha and beta diversity. Yield and associated parameters (number of grown and fallen/broken plants, rating fence, plant vigor, the occurrence of Ustilago sp., and grain moisture) were evaluated after harvest.

RESULTS

Seeds exibited lower bacterial diversity compared to the soil, root, and manure samples.The most abundant taxon in uninoculated seeds pre-harvest was Pantoea, while in seeds treated with PHY the most abundant was Acinetobacter, followed by Pantoea, Pseudomonas, and Bacillus. After harvest, Pantoea and Pseudomonas prevearled in seeds. Soil bacterial communities mostly remained unchanged, regardless of the treatment (PHY, PM, and PHY_PM) applied or the tested phenophase, with uncultured Gaiellales and Bacillus being the most abundant. Contrarily, root bacterial communities differed in distribution and relative abundance

of different taxa between phenophases and between treatments. The most abundant taxa in roots during the inial phenophase (II) was Pseudomonas. In the flowering phenophase (III), Bacillus prevealed with two to three times higher relative abundance in treatments with PHY or PM compared to the negative control, while Lechevalieria dominated in harvesting phenophase (IV). A statistically significant increase in maize yield was obtained in the treatment with PHY, with an average value of 650 kg/ha compared to the negative control. The lowest yield was obtained in the treatment with PM.

DISCUSSION

The prevalence of Acinetobacter, Pantoea, Pseudomonas, and Bacillus in seeds treated with PHY pre-harvest, indicates that treatment with PHY is highly beneficial considering the known plant growth promoting potential of these genera, that were also previously confirmed as core maize inhabitants (Mehta et al., 2021). As core members, Pantoea and Pseudomonas remained present after harvest. The benefit of the application of Bacillus-based fertilizers to soil is the enhancement of the plant-available forms of nutrients and the inducement of pest and pathogens defense systems (Radhakrishnan, et al., 2017). It is of crucial importance that none of the three treatments applied in this study affected the composition of the indigenous soil bacterial communities during four tested phenophases, which is highly important when selecting suitable agricultural practices. Shifts in root microbiome over maize growth could be related to the production of different root metabolites over the growing season (Bourceret et al., 2022). Roots were especially rich with genera (Pseudomonas, Stenotrophomonas, Sphingobacterium, Achromobacter) known as phosphate solubilizers (Mehta et al., 2021). Furthermore, Bacillus was dominant in roots in flowering phenophase. This genus is known for its wide spectrum of beneficial effects on plants, like phosphate solubilization, biosynthesis of growth hormones, antimicrobial activity, induction of systemic resistance, etc. (Dimkic et al., 2022).

CONCLUSIONS

Considering the above-mentioned effect of PHY on maize yield incensement, its non-disruption effect on the core microbiome, and the positive effect on enhancing the presence of beneficial bacterial genera, this microbial inoculant could be proposed as a promising alternative to chemicals and organic fertilizers in maize cultivation.

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PARTICULATE MATTER CLEANING THROUGH SELF-ASSEMBLED CALCIUM CARBONATE PARTICLE ARRAYS

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INTRODUCTION

Employing nanoparticles in a wide variety of applications, including air filtration, is promising. Air pollution has a significant impact on the health of the global population, the ecology, and climate change, causing up to 7 million premature deaths annually. [1] Particulate matter (PM), which when inhaled travels deep into the lungs and/or bloodstream, is the main culprit. [2]

STATE OF THE ART

Filtration and reducing pollution at the source, ozone oxidation, plasma air purification, and vegetation are examples of potential remedies to reduce PM in the air. Filtration is a mechanical procedure that removes PM from the air by providing a barrier through which only clean air may pass. [3] Indoor PM reduction can also be accomplished with the use of ozone-oxidation and plasma air purification technology. [4] Vegetation canopies are another option, as they may absorb both gaseous and particle pollution. [5,6]

RESULTS AND DISCUSSION

Here, we suggest a unique approach to removing PM from the air by using self-assembled arrays of micro- and nano-sized CaCO3 particles. Fabricating thin coatings of a non-volatile component on solid substrates using evaporative spin-casting is a widespread procedure [3], while drop coating is another effective technique for coating and activating a solid surface.

The self-assembled arrays are subjected to polluted air to begin the air-cleaning process, which is followed by the adsorption of PM onto the porous CaCO3 microparticle arrays. Atomic force microscopy has been used to study the stability of the system in environmental conditions. The results demonstrated the ability of self-assembled CaCO3 microparticles to adsorb PM from the air in both environmental and laboratory settings.

CONCLUSIONS

In conclusion, our research demonstrates that the self-assembly process is an inexpensive and feasible way to prepare such structures. CaCO3 particles are an excellent choice for applications in air purification because they are recyclable and have the potential for industrial-scale production. Adsorbed particulate matter on self-assembled CaCO3 particles can be recovered and recycled, if necessary.

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NATURAL FRACTIONATION OF MICROALGAE AND CYANOBACTERIA AS A METHOD FOR HYDROGEN ISOTOPE SEPARATION

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INTRODUCTION

Fractionation is a process in which there are preferences for consuming lighter isotopes compared to heavier ones. Algae and cyanobacteria are photosynthetic organisms splitting water to hydrogen and oxygen. Enzymes contributing to this prefer lighter hydrogen isotope to the heavier one, i.e. tritium. In addition, these organisms have hydrogenases which can produce hydrogen. Cyanobacteria have also nitrogen fixation features. Therefore, we assess the possibility of using these biochemical processes to separate tritium and hydrogen through natural fractionation on one hand and produce hydrogen (lighter isotope) on the other hand. Through this process, level of tritium will increase in the water and biomass. We hypothesize that this induced hydrogen fractionation would be sufficient as a biotechnical method to remove tritium from wastewater. For this purpose, we selected two strains of cyanobacteria (Synechococcus elongatus and Synechococcus leopoliensis) and a species of microalgae (Chlorella sorokiniana). At first stage, growth rate was measured using optical density. After this, growth conditions including temperature, light and media conditions will be assessed. Tritium separation was also measured using liquid scintillation counting (LSC).

STATE OF THE ART

Due to the chemical similarity of hydrogen and tritium, their extraction and purification has relied for decades on expensive and energy-demanding processes [12]. In addition, most of these processes produce toxic and harmful by-products like sulfide gases and alkaline solutions used for electrolysis [13]. In this work, a natural process is proposed to separate the isotopes; therefore we expect high efficiency and the least harmful by-products. In addition, hydrogen can be produced. Therefore, during this process, tritium-polluted water is purified and hydrogen is produced. And based on literature review, this method has never been used for tritium enrichment.

RESULTS

Initial results in the laboratory scale conditions show that the cyanobacteria have grown on BG-11 medium in incubator at 37°C and under white LED light. They grew on agar plates and for the liquid media, growth was observed after pH adjustment. On the other hand, algae put in TAP medium under blue-violet LED light at room. Optical density (OD) results indicate a 14-day period until stationary growth level. Liquid scintillation counting (LSC) results also suggest some enrichment, especially under nitrogen for cyanobacteria.

DISCUSSION

Based on the initial results, this method could be used for tritium enrichment. However, the growth conditions should be optimized to increase the growth rate. Temperature, light strength

and duration, pH, and stirring need to be optimized at next phases. In addition, experiments should be calibrated to get high fractionation efficiency.

CONCLUSIONS

Microalgae and bacteria split water during photosynthesis, like electrolysis. Enzyme preferences for lighter isotopes would lead to isotope fractionation. In this work, this biochemical process is used for tritium enrichment. In addition, it is stated that hydrogen can be produced by hydrogenases. Results show that this process can be used for this purpose but it needs some optimization.

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ORGANOMERCURIAL LYASE (MERB) ENABLED METHYLMERCURY DETECTION

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Mercury is a highly toxic and mobile element that has had a pronounced and adverse effect on organisms. Accordingly, bacteria have evolved mer operons to meliorate toxic action of different chemical forms of mercury. The bacterial mercury detoxification system contains two proteins, organomercurial lyase (MerB) and mercuric ion reductase (MerA). MerB specifically catalyzes the protonolysis of the carbon-mercury bond of methylmercury (MeHg), resulting in the formation of a reduced carbon compound and inorganic ionic mercury (Hg2+) [1]. A MerBADtt complex consisting of the MerB, a mercuric ion, a dithiothreitol (DTT) and mercuric reductase MerA is then releasing Hg0 (elemental mercury) [2]. Since MerB is a highly specific enzyme, we are planning to use its Met-Hg specific binding characteristics as a sensing component of the sensor. The formed complex of MerBADtt can act as a transducer if combined with precise Hg0 measurement. In order to achieve that, we have prepared an expression system that will enable us to obtain a high enough amount of MerB and MerA enzymes. The expressed MerB and MerA enzymes with his-tag would be purified and evaluated for their use in the preparation of the Met-Hg specific sensor. The obtained MerB activity is going to be determined through MeHg binding and conversion to Hg2+ using cold vapour atomic fluorescence spectroscopy (CVAFS).

According to the enzymatic activity of the complex, we also hypothesize that the release of Hg from the MerBADtt-Hg can also be monitored in real-time by following the released Hg0 as a product, which can be captured or measured using the Lumex mercury analyzer. By using the MerBADtt complex, we are planning to determine the sensitivity, durability and specificity of this approach in real-time using various environmental matrices.

According to our current level of the development of the methods we welcome participants of the workshop to discuss about our approach.

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RADIOLABELLING OF NANOPARTICLES FOR COLLOID TRACING AS A VERSATILE TOOL IN NANOSAFETY RESEARCH

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INTRODUCTION

Accurate quantification of nanoparticles (NPs) in complex media remains a considerable challenge when assessing the risk that manufactured nanoparticles pose for humans and environment. The radiolabelling of nanoparticles is a valuable tool for conducting lab-studies with realistic systems and realistically low NP concentrations. We present an overview of our radiolabelling efforts with examples of their applications

RESULTS

We have developed various methods of introducing radiotracers into some of the most common NPs, such as Ag, carbon, SiO_2, CeO_2 and TiO_2 NPs. The labelling techniques are the synthesis of the NPs using radioactive starting materials [1], the binding of the radiotracer to the NPs [2], the activation of the NPs using proton irradiation [3], the recoil labelling utilizing the recoil of a nuclear reaction to implant a radiotracer into the NP [4], and the in-diffusion of radiotracers into the NPs at elevated temperatures [5]. Using these methods we have produced [Ag-105/110m]Ag, [I-124/125/131]CNTs, [V-48]TiO_2, [Ce-139/141]CeO_2, [Be-7]MWCNT, [Cu-64]SiO_2, [Ti-44/45]TiO_2, etc. for accurate quantification in complex media at environmentally relevant low concentrations.

The NPs labelled by our methods can be detected at minimal concentrations well in the ng/L range even with a background of the same element or other nanoparticulate matter and without complicated sample preparations necessary. The methods are adaptable for a wide range of other NPs. The labelled particles have been successfully used in release studies, environmental mobility studies, fate studies in waste water treatment and plant uptake studies and could provide a valuable tool for accurate dose measurements in toxicity studies.

SUMMARY

A library of radiolabelling strategies for the most common nanoparticles was developed that allows accurate detection at low concentrations in complex media.

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ISOLATION OF MCPA-DEGRADING ENDOPHYTIC BACTERIA FROM CUCURBITS

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INTRODUCTION

MCPA (2-methyl-4-chlorophenoxyacetic acid) is one of the most commonly used herbicide active substance to selectively control annual and perennial weeds in agriculture. There is still limited monitoring data, however recent reports showed that MCPA can be accumulated in high concentrations in bottom sediments. Thus, there is an urgent need to find methods which will prevent spread of these herbicides into untargeted areas. MCPA removal occurs inter alia as an effect of biodegradation, especially by bacteria harboring the functional tfdA-like genes, involved in the first step of phenoxy acids degradation pathway. A promising tool for MCPA removal from soil ecosystems lies in the integration of phyto- and bioremediation due to existing interactions between plant roots, root exudates, soil and plant-associated microorganisms. Our previous research showed that the amendment of soil with plant secondary metabolites (PSMs), which are structurally similar to selected pollutants (i.e. MCPA) and simultaneous cultivation of zucchini is a promising way to enhance the removal of such herbicides from the environment. We previously showed that a characteristic PSM for cucurbits i.e. syringic acid (SA) mitigates the toxic effect of MCPA in soil (Mierzejewska, Tołoczko, et al., 2022). Additionally SA enhances the occurrence of MCPA-degrading tfdA-like genes and improves the removal of MCPA (Mierzejewska et al., 2019). Our previous research showed also that the SA-amendment of soil contaminated with MCPA can shape the endosphere microbiome of cucurbits (especially leaves) (Mierzejewska, Urbaniak, et al., 2022).

STATE OF THE ART

Until now little has been known about the presence and the role of endophytic bacteria in MCPA biodegradation. Bacteria utilizing MCPA have been isolated from soil, however there is no research which states that endophytic bacteria are able to use MCPA as a sole carbon source. Therefore, the main aim of this research was to isolate bacteria from plant tissues (leaves and roots), which can grow on MCPA-enriched medium and use up MCPA as a sole carbon source. Additionally, we studied the differences between the composition of isolated strains from MCPA-contaminanted soil or/and amended with SA.

RESULTS

From 284+C medium enriched with MCPA we isolated endophytic bacteria belonging to families: Bacillaceae, Erwiniaceae, Micrococcaceae, Jonesiaceae, Nocardiaceae, Microbacteriaceae, Paenibacillaceae, Enterobacteriaceae, Xanthomonadaceae, Pseudomonadaceae, Alcaligenaceae, Rhizobiaceae and Caulobacteraceae. Additionally, plant growth-promoting tests showed that some of the isolated strains can support plant growth under stress conditions by: ACC-deaminase production, IAA growth hormone and organic acids production. Further investigation verified strains which can solely use MCPA as carbon source i.e. Pseudomonas

monteilii; Pseudomonas sp. 12B_3; Pantoea agglomerans; Paenarthrobacter nicotinovorans and Acinetobacter johnsonii.

DISCUSSION

Literature showed that cucurbits and their associated bacteria can significantly enhance the removal of persistent organic contaminants from soil (Eevers et al., 2018). Wang et al. (2021) showed that endophytic fungi strain Phomopsis sp. can significantly enhance the biodegradation of MCPA in soil. Also Germaine et al. (2006) showed that Pseudomonas putida, endophytic bacteria isolated from poplars, can contribute to better removal of 2,4-D (2,4-dichlorphenoxyacetic acid) from studied medium. Our previous results (Mierzejewska, Urbaniak, et al., 2022) showed that the amendment of soil with phenolic compounds significantly influences the structure of leaves endosphere. What is more, we showed that presence of some potential MCPA-degraders both in soil and plant endosphere can be enhanced by the addition of SA. Cultivation-dependent approach confirmed previous observations: our results for the first time confirmed the presence of endophytic bacteria, which can tolerate and grow on MCPA-enriched media, in dicotylendonous plants (cucurbits). Additionally, the amendment of soil with PSM (SA) influenced the composition of bacterial structure in studied plants tissues. Selected strains (Pseudomonas monteilii; Pseudomonas sp. 12B_3; Pantoea agglomerans; Paenarthrobacter nicotinovorans and Acinetobacter johnsonii) will be subjected to further research on the expression of functional genes responsible for crucial steps of MCPA degradation.

CONCLUSIONS

Our results confirmed presence of bacterial endophytes which can tolerate and use up MCPA as a sole carbon source. Additionally, their plant growth promoting properties were verified. Selected strains will be further used for the continuation of study on the effect of selected PSMs on MCPA degradation. Additionally, their effectiveness in endophyte-enhanced phytoremediation with the use of cucurbits as multipurpose plants will be studied.

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INVESTIGATION OF URANIUM(VI) REDUCTION BY THE REPOSITORY-RELEVANT BACTERIUM DESULFOSPOROSINUS HIPPEI DSM 8344T

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INTRODUCTION

For a comprehensive safety assessment regarding the deep geological disposal of high-level radioactive waste, various aspects have to be taken into account. Besides geological, geochemical, and geophysical properties, the influence of naturally occurring microorganisms in the surrounding host rock and backfill material play a crucial role in the environment of such a repository. Clay formations are potential host rocks for the long-term storage of this waste, whereas bentonites are supposed to serve as backfill material, not only for a final disposal site in clay formations but also in crystalline rock. In the event of a worst-case scenario, if water enters the disposal site, radionuclides can escape from the waste canisters and thus interact with the microorganisms. This can, for example, lead to changes in the chemical speciation or the oxidation state of the metal ions.

RESULTS & DISCUSSION

Under repository-relevant conditions, Desulfosporosinus spp. are important representatives of anaerobic, sulfate-reducing bacteria being present in clay formations as well as in bentonites. Various studies show that they are playing a major role in the microbial communities of these surroundings. A closely related microorganism to the isolated species is Desulfosporosinus hippei DSM 8344T. Therefore, this bacterium was used to investigate its interactions with uranium(VI) especially regarding the reduction to the less-mobile uranium(IV) having favorable properties like a reduced mobility.

Time-dependent reduction experiments showed the removal of about 80% of the uranium(VI) from the supernatants in artificial Opalinus Clay pore water (100 µM uranium(VI), pH 5.5) within 48 h. Corresponding UV/Vis measurements of the dissolved cell pellets provide clear proof of the formed uranium(IV). The proportion of this oxidation state in the cell-bound uranium increases up to 40% after one week. Therefore, a combined sorption-reduction process is a possible interaction mechanism.

Time-resolved laser-induced luminescence spectroscopy reveals the presence of two uranium(VI) species in the supernatant. A comparison with reference spectra leads to an assignment to a uranyl(VI) lactate and a uranyl(VI) carbonate complex. The species distribution shows a decrease of the proportion of the lactate species with time, whereas the proportion of the carbonate species remains almost constant.

Uranium aggregates are formed on the cell surface during the process, as determined by scanning transmission electron microscopy (STEM). Furthermore, cells release uranium-containing vesicles as a possible defense mechanism against cell encrustation.

CONCLUSIONS

The findings of this study help to close existing gaps in a comprehensive safeguards concept for a repository for high-level radioactive waste in clay rock. Moreover, this study provides new insights into the interactions of sulfate-reducing microorganisms with uranium(VI) and thus, contributes to new bioremediation approaches of radionuclide-contaminated environments, as well.

PSEUDOMONAS SPP. IN BIOCONTROL OF CROWN GALL DISEASE: NEW APPROACHES

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INTRODUCTION

Crown gall is an economically important and widespread plant disease caused by tumorigenic bacteria that are commonly affiliated with the genera Agrobacterium, Allorhizobium and Rhizobium. Novel and an atypical group of tumorigenic agrobacteria belonging to the genus Rhizobium ("tumorigenes" clade) was identified as a causative agent of crown/cane gall on blackberry, rhododendron and blueberry in Serbia and Germany (Kuzmanović et al., 2018 and 2019). Efficient measures to control crown gall disease were not reported till nowadays, so assessment and application of alternative biological control measures would contribute to sustainable agricultural production and environmental protection. The aims of the study were 1) identification of candidate bacterial strains that could be employed for biological control 2) to analyse phytobiome of the treated and non-treated crops and 3) to perform a whole-genome sequencing of a few most promising biocontrol strains.

STATE OF THE ART

Antimicrobial activity of ten biocontrol candidates from rhododendron and 27 additional antagonistic strains were tested in vitro against the tumor-inducing strain Rhizobium sp. rho-6.2. The six most efficient Pseudomonas and Bacillus strains were tested in vivo, using co-inoculation and preventive inoculation strategies in controlled greenhouse conditions on tomato plants as a model system in four replicas and randomized. Tumors from the most effective treatments were sampled, and then total DNA was isolated and subjected to the next-generation sequencing (NGS). Direct analysis of bacterial communities using Illumina MiSeq sequencing of 16S rRNA gene amplicon libraries was performed to assess the microbial ecological effect, with complete bioinformatic and computational biology analysis conducted. Also, a whole-genome sequencing of a few most promising antagonistic strains was performed.

RESULTS

Among six antagonistic strains, the most efficient in co-inoculation strategy against pathogenic Rhizobium sp. rho-6.2 were two Pseudomonas strains (R-6.10 and R-11.20), which reduced a tumor size 92.86%. The same Pseudomonas strains were less effective in preventive treatments (15.38 and 30.77%). Although Bacillus strains exhibited high in vitro antimicrobial activity, their in vivo activity was in preventive treatment only 15.38%, whilst in co-inoculation strategy was detected as moderate (42.86%). Bacillus and Pseudomonas strains applied together increased biocontrol activity with 38.6% of tumor's reduction. In analyzed treatments, was detected the dominant presence of Proteobacteria followed by a moderate presence of Actinobacteriota and Firmicutes. On the genus level, the most abundant, both in negative control and treatments, were representatives of Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium group (18,53% - 71,81%) followed by Pseudomonas spp. (2,76%- 36,46%). According to alpha diversity indexes

on the genus level, the highest values were detected in the negative control, pre-treatment with Pseudomonas sp. R-6.10, co-inoculation with Pseudomonas spp. R-6.10 and R-11.20 individually. Analysis of beta diversity by the DPCoA matrix exhibited that the co-inoculation and positive control groups were well separated, whilst preventive treatment overlapped both the co-inoculation and positive control samples. Differential abundance analysis on a genus level revealed a statistically higher presence of Stenotrophomonas and Asanoa in preventive treatments and Dyadobacter and Pandoraea spp. in their positive control. In the co-inoculation strategy, Pseudolabrys and Asanoa were prevalent in treatments and Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium was detected as prevalent in positive control. Whole-genome sequencing and preliminary comparative genomics analyses revealed that the best biocontrol candidates, Pseudomonas strains R-6.10 and R-11.20 represent two new species, most closely related to P. graminis and P. fildesensis, respectively.

DISCUSSION

The Pseudomonas species exhibited the most prominent activity in vivo. Pseudomonas genus is rich in species with the potential for biocontrol of wide spectra of pathogens. Their activity is based on the production of variety of antimicrobial compounds (Dimkic et al., 2022). Also, silencing quorum sensing or quorum quenching is one of their biocontrol strategies by attenuating the virulence of the pathogen (Zhang et al., 2021). Metabarcoding analysis showed differences between treatments, mainly on the level of less presented genera. Best candidates for biocontrol of crown gall, Pseudomonas spp. R-6.10 and R-11.20 originating from the crown gall tumor, confirms the previously established hypothesis that plants are the best sources of biocontrol agents (Janisiewicz et al., 2013).

CONCLUSIONS

The selected Pseudomonas strains could be further tested as an alternative strategy for the biocontrol of crown gall disease and the potential involvement of the quorum quenching mechanism will be determined. Crown gall tumors have shown to be a great source of antagonistic isolates Pseudomonas sp. R-6.10 and Pseudomonas sp. R-11.20 identified according to WGS as the two new species that further needs to be described.

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GREEN SOLUTION FOR THE HEAVY PROBLEM: SPATIALLY-ORIENTED ARTIFICIAL STRUCTURES MADE OF DISSIMILATORY METAL-REDUCING BACTERIA ARE ABLE TO PRECIPITATE VANADIUM AEROBICALLY

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INTRODUCTION

Currently, the most of treatments of polluted sites are based on lowering the amount of the pollutant either by removal, dissolution, or degradation processes. However, recalcitrant organic substances like lignin or polyaromatic hydrocarbons from paper industry and oil spills can be also a very useful source of new fine chemicals if the pollutant is properly decomposed. In addition in the future, there is going to be increasing demand for the recycling of inorganic toxic metals as an example from the battery-producing industry. In addition, the removal of inorganic substances such as toxic metals like lead, cadmium, uranium, or vanadium from main tailing effluents can be also a useful source of nanoparticles or catalysts. However, for both cases, either organic or inorganic pollutants, there are no available cheap and efficient technologies. Here, we are proposing the use of multispecies biocatalytic structures formed by the aggregated microbial strains where different microniches are formed in order to either induce intercellular cross-feeding metabolic cascades or forming environmental conditions that can induce precipitation of inorganic ions. As an example, we used dissimilatory metal-reducing bacteria (DMRB) where most of the known DMRB strains are facultative or obligate anaerobic organisms, which imposes infrastructural restrictions on their use in biotechnology. Environmental factors like oxygen concentration could drive to the formation of anaerobic zones within the biofilms or bacterial flocks, so multispecies bacterial communities could work as an effective factory for metal reduction and decomposition of complex organic molecules. Manipulating bacterial communities through the spatial distribution of community members can greatly facilitate above mentioned biotechnological processes and make them efficient and inexpensive.

STATE OF THE ART

To our knowledge, no methods of controlling the processes of construction of bacterial niches have been published so far. Hence, here we are showing a novel approach for the construction of spatially oriented bacterial communities using an electrostatic modification of bacterial cell surface (Rybkin et al., 2019). As a proof of concept, cells of well-known DMRB Shewanella oneidensis were used for the construction of artificial aggregates and its characterization of

dynamics of such structures and its ability to use an anaerobic pathway for the reduction of vanadium, forming micro and nanoparticles, while cultivated aerobically.

RESULTS

Estimation of the robustness of constructed aggregates showed its ability to keep the form for up to 10 days in stationary cultivation and up to 5 days shaking at 150 rpm speed. Indirect evidence of the formation of anaerobic cores was obtained from the observations of solid particles in the center of constructed aggregates using differential contrast light microscopy (DIC). More detailed characterization of formed vanadium particles 0.5-1µm size was confirmed via Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDS). Quantitative estimation of anaerobic activity of multicellular aggregates by diphenylcarbazide assay (Carpentier et al., 2003) showed the reduction activity of 14.5±1.06 µM per µg of protein biomass which is 4.75 times higher than it was determined within the planktonic fraction of cells ($p<0.05$).

DISCUSSION

The developed method showed great potential for many biotechnological applications since DMRB like Shewanella could be applied for different pollutants due to its high versatility of metabolic pathways. At the same time, it should also be kept in mind that for any further used strains, the protocol of electrostatic modification must be adjusted since individual parameters of cells like morphology or polyelectrolyte tolerance play a crucial role in the stability of formed aggregates. Although the first experiments clearly showed the possibility to form anaerobic niches in aerated conditions, mechanisms behind these processes as well as physiological adaptations of strains in aggregated forms remain unknown and require to be studied in the future.

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ISOLATION, DIVERSITY AND CHARACTERIZATION OF PLANT GROWTH-PROMOTING BACTERIA FROM FIVE DIFFERENT SUGAR BEET HYBRIDS

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INTRODUCTION

Plant growth promoting bacteria (PGPB) are beneficial bacteria that can stimulate plant growth through various mechanisms such as nitrogen fixation, phosphate solubilization, production of siderophores, indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Furthermore, PGPBs are recognized for the protection of host plants against phytopathogens via different mechanisms that involve the production of antimicrobial components, hydrolytic enzymes, competition for nutrients and induction of resistance in the host plant (Dimkić et al., 2022). Global food security is greatly challenged by emerging pathogens and climate change facing rising temperatures, extreme weather events and long periods of drought (Sessitsch et al., 2018). Sugar beet is the most important crop for sugar production in temperate zones, while it is the second most important in the world and covers 30% of the world's sugar production. Unfortunately, sugar beet production is exposed to several phytosanitary problems which include various fungal pathogens resulting in significant losses in crop yield and quality (Farhaoui et al., 2022).

STATE OF THE ART

Root, seed and rhizosphere samples from five different sugar beet hybrids [Eduarda (ED), Tibor (T), Tajfun (TF), Koala (KO) and Cercospora-resistant (C)] were used for the isolation of plant-associated bacteria. Different formulations of nitrogen-free selective media were used for the isolation of diazotrophic bacteria. The isolates were tested in in vitro experiments for plant growth promotion (nitrogen fixation, phosphate solubilization, production of extracellular polymeric substances (EPS), swarming and swimming ability, production of IAA, hydrogen cyanide (HCN), ACC deaminase and siderophores), production of extracellular enzymes, drought and salinity tolerance and antifungal potential against Cercospora beticola and 21 different plant pathogenic strains of Fusarim spp. The extraction of ultra-pure DNA of the seeds was done using the Zymo BIOMICS DNA Mini Kit, 16S library preparation, using Nextera XT Index Kit, and amplicon sequencing step was performed using a 2×300 bp paired-end run on a MiSeq Sequencer, according to manufacturer's instructions (Illumina) in commercially available service.

RESULTS

A total of 156 isolates were obtained from all hybrids, including 14, 27 and 49 unique species from the rhizosphere, roots and seeds, respectively. The genera Bacillus and Lysinibacillus were characterized the rhizosphere of all hybrids; without similarities observed between isolates from the roots, while B. subtilis was unique as a common species for all seeds. With a total of 93 strains, the seeds were the most abundant with different bacterial species, especially for the ED, KO, and C hybrids. In general, the roots had abundant species of Paenibacillus, Curtobacterium, Mycetocola, Knoellia, Neorhizobium, Microbacterium, Rhodococcus, Massilia, Rathaiibacter.

Species from the genera Bacillus, Lisinibacillus, Kocuria, Sanguibacter, Pantoea, Glutamicibacter, Pseudomonas, Erwinia, Providencia and Pseudoclavibacter were prominent as seed endophytes. Metabarcoding analyzes revealed that the phylum Proteobacteria was the most dominant in all seeds of all five hybrids followed by Cyanobacteria, Actinobacteriota, Firmicutes Bacteroidota and Chloroflexi. TF hybrid differed significantly in bacterial diversity from other hybrids with the highest abundance of Firmicutes. Higher alpha diversity was observed in ED, KO and T at all taxa levels compared to C and TF hybrid. This was in correlation with estimated and observed richness. The lowest richness was characterized TF hybrid at all taxa levels. The most prevalent genus was Pantoea especially in the C, ED and TF hybrids. Pseudomonas was the second most abundant genus in all hybrids, except for TF. Weissella was only detected in the TF hybrid with relative abundance (RA) of 7.14%, while Enterobacter, Enterococcus, Staphylococcus and Erwinia were the most abundant. Gardnerella (4.88%), Glutamicibacter (2.28%) and Prevotella (2.02%) were found exclusively in KO hybrid. Most dominant genera in T hybrid were Pantoea, Pseudomonas, and Acinetobacter along with Actinomycetospora and Streptococcus considering other hybrids. C hybrid was the poorest in diversity and it was dominated by Pantoea, Kosakonia and Pseudomonas. Also, Pseudoclavibacter was only detected in C hybrid (1.07%). Most detected genera in ED were Pantoea, Pseudomonas, Gilliamella and Lactobacillus. Initial selection from 156 isolates highlighted 32 isolates, which all of them had the ability to grow on nitrogen-free media while the vast majority had the ability to solubilize phosphates, produce siderophores, IAA and EPSs. HCN production was recorded for nine isolates, while only two ACC deaminase producers were detected. An 11 isolates successfully tolerated high concentrations of salt (10%), while 20 isolates successfully grew at the maximum concentration of PEG (30%), of which seven were highly tolerant to drought conditions. The most frequently secreted enzymes were lipase and gelatinase, followed by amylase, proteinase, pectinase, cellulase and mannanase. The xylanolytic activity was recorded for six isolates. Selected isolates successfully inhibited the growth of all tested fungi, except for Fusarium bothi IB7I-1, which was the most resistant strain.

DISCUSSION

Looking at the diversity of culturable bacteria of the rhizosphere, roots and seeds of each hybrid separately, it can be observed that there was almost no overlap in species. This is proof that plant genotypes drive the microbiome composition. The importance of plant growth-promoting bacteria is already known to the scientific community. PGPB isolated from different crops already showed good promise in using these inoculants as biofertilizers and biocontrol agents. Bacteria isolates from different sugar beet hybrids in this research belong to the genera known for PGP abilities (Souza et al., 2015), and more research will be conducted in the future on them.

CONCLUSIONS

Seeds, roots and rhizosphere of sugar beet hybrids differ significantly in bacterial diversity. The culturable approach showed that the seeds of ED and KO hybrids are the most bacterially abundant, which was also confirmed by the non-cultivable approach. The most potent isolates, C3-16/2.1, C3-19 (Bacillus amyloliquefaciens), KO3-18 (B. subtilis), T2-23 (Bacillus velezensis), C3-36, KO3-44 (Mixta theicola), ED2-6 (Curtobacterium pusillum) and KO3-19 (Microbacterium saccharophilum), showed excellent characteristics for plant-growth promotion and antifungal potential. From the perspective of future research, these isolates will be used in the design of bacterial consortia which will be applied in planta.

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NEW ALL-NANOPARTICLE MICROCAPSULES FOR REMOTE RELEASE AND SENSING

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Abstract Typically, LbL-assembled microcapsules have been formulated via alternating deposition of positively and negatively charged polyelectrolytes onto sacrificial templates or so-called cores. In this work, we extend the LbL assembly technique to produce microcapsules solely based on nanoparticles instead of polymers. Both gold and silver nanoparticles have been deposited as oppositely charged layers in the LbL assembly process. We have identified the minimum number of layers by scanning electron microscope (SEM) for assembly of capsules on calcium carbonate templates. X-ray diffraction (XRD) studies confirmed the dissolution of calcium carbonate, while mechanical properties of such capsules probed by atomic force microscopy (AFM) reveal an essential increase in the stiffness and density in the walls. Dual functionality of such new capsules has been achieved by actions of ultrasound and laser. Since ultrasound acts more effectively on denser objects, low intensity (below 1 W/cm2) of ultrasound has been used to enable release of encapsulated content. Laser has been used to illuminate microcapsules located in cells and effective killing of cells was achieved. Subsequently, the composite capsules comprised of metallic nanoparticles were applied as Surface Enhanced Raman Scattering (SERS) platform, where the performance of purely nanoparticle-based shell of capsules is envisioned to enable SERS either of solutes or macromolecular structures such as bacterial cells. Further applications of the assembled microcapsules are expected in conducting the rapid diagnostics, catalytic reactions and biomedicine.

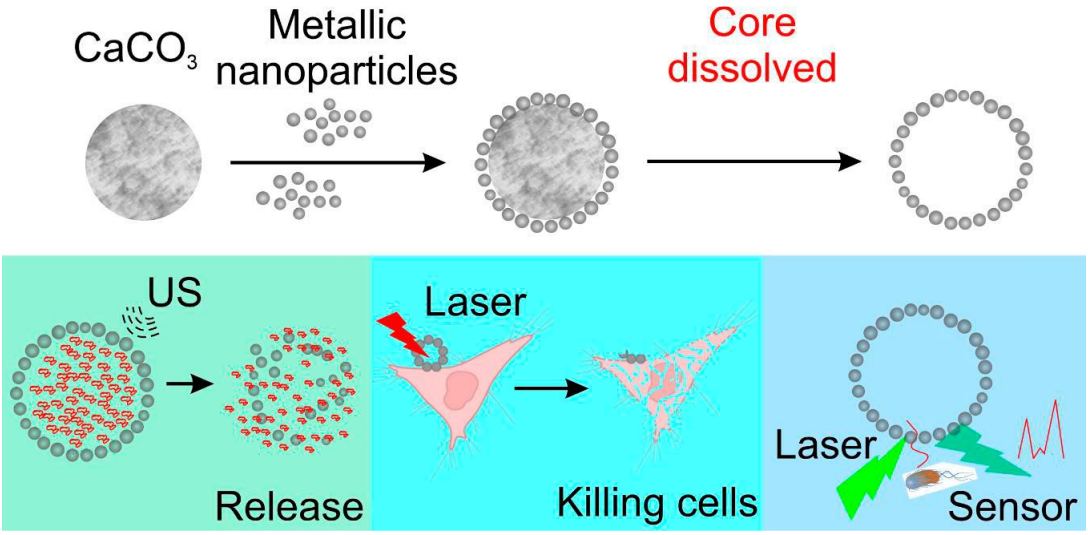

Figure 1. Schematics of the work showing major steps employed for the work of all-nanoparticle capsules.

LC-MS/MS DETERMINATION OF THE PRODUCTS OF BACTERIAL LIGNIN DEGRADATION

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Lignin is a complex heterogeneous aromatic biopolymer, found in plant cell walls as one of the components of lignocellulose [1]. Though primarily present in nature, large quantities are daily generated as a waste product in paper and biofuel industries. Lignin is a rich renewable source of aromatic molecules, but despite its considerable potential for transformation into value-added chemicals and fuels, it is still considered a waste product, serving as low-quality fuel [2]. This is mainly due to its heterogeneous composition and resistance to degradation, which makes any refinement very complex. Moreover, the obtained products require extensive separation and purification [3].

The recent energy crisis has however led to a rising interest in lignin and its valorization, particularly the environmentally friendlier degradation employing fungi and bacteria, who have developed ligninolytic enzymatic systems. These could potentially be exploited for degradation of waste lignin, generating value-added products. The majority of research has so far been done on fungi, initially considered to be more promising due to higher lignin-degrading capacity, but later proving unsuitable for large-scale use due to the complexity of their enzymes [4].

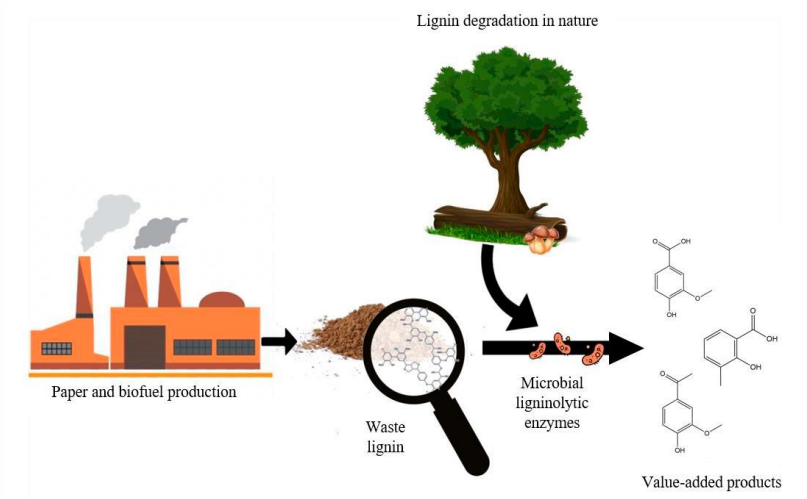

Figure 1: The idea of using naturally present mechanisms to valorize waste lignin

We studied the less researched bacterial degradation, focusing on selected strains of the species Paraburkholderia jirisanensis, which hold a gene for the production of ligninolytic enzymes of the laccase class. Analyses comprising target and non-targeted LC-MS/MS and LC-HRMS screening were performed on the product mix, formed during the incubation of aforementioned bacteria with lignin as its energy source. The target analytical method was developed using 14 monomeric phenolic compounds that have been reported in the literature as degradation products, four of which were later detected and quantified in the product mixture. In addition to that, tentative structures of further compounds have been proposed by a non-targeted approach via comparison with HRMS databases.

Developed target method for simultaneous detection of monomeric degradation product candidates presents a simple and fast screening tool that can support the studies of ligninolysis by bacteria. Results, obtained for studied strains of Paraburkholderia jirisanensis sp. by targeted as well as non-targeted screening approach both clearly demonstrate their ability to degrade lignin, therefore holding a potential for environmentally friendly lignin valorization.

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INCORPORATION OF NONCANONICAL AMINO ACIDS INTO PROTEINS USING GENETIC CODE EXPANSION

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INTRODUCTION

The use of genetic code expansion (GCE) enables the incorporation of over 200 non-canonical amino acids (ncAAs) into target proteins (Vargas-Rodriguez et al., 2018). The side chains of ncAAs are chemically and structurally diverse and include selectively reactive groups, spectroscopic probes, photoreactive groups, stabilizing large hydrophobic or aromatic groups and other (Dumas et al., 2015). Their applications range from improving stability of engineered proteins (Ohtake et al., 2015) and creation of avirulent viruses to creation of semi-synthetic organisms and their biocontainment (Vargas-Rodriguez et al., 2018).

The incorporation of ncAA into a target protein relies on the reassignment of one of the codons to the ncAA, which is achieved by the expression of an orthogonal aminoacyl-tRNA synthetase (o-aaRS) and its cognate tRNA. For the introduced aaRS•tRNA pair to be orthogonal, the aaRS should not acylate natural tRNAs with the ncAA or orthogonal tRNA with canonical amino acids, while the orthogonal tRNA should not be acylated by the aaRSs of the host (Vargas-Rodriguez et al., 2018). Finally, the ncAA itself should not be modified or degraded by cellular enzymes.

STATE OF THE ART

To incorporate the ncAA site-specifically into a target protein, the desired position in its gene must first be changed to the codon that is to be reassigned to the ncAA. The codons that are most commonly reassigned are the stop codons, as they are not recognized by the tRNAs acylated with natural amino acids (Vargas-Rodriguez et al., 2018). The target gene with a stop codon and the o-aaRS•tRNA pair are then introduced into a suitable organism or cell line (Vargas-Rodriguez et al., 2018). Genes of the target protein and the o-aaRS are expressed in the organism from the separate inducible promoters to reduce the toxicity of the orthogonal translational system that may suppress stop codons of the non-target proteins, and to allow optimal control of target protein expression. To maximize the yield of the target protein, expression of o-aaRS is first induced to accumulate the ncAA-tRNA at concentrations high enough to compete with the release factor, which also binds to the stop codon, and finally expression of the target protein is induced.

RESULTS

We use the orthogonal aaRS•tRNA systems to produce superstable protein pores for use in nanopore biosensing by introducing m-Cl-tyrosine (m-Cl-Tyr) and p-pentafluorosulfanyl-phenylalanine (SF5-Phe) at the interfaces between protomers of lysenin and actinoporin pores.

To date, we have constructed plasmids containing pore forming protein genes with internal TAG stop codons, inserted them into Escherichia coli and co-expressed them with the tyrosyl-tRNA synthetase and tRNATyr from Methanocaldococcus jannaschii that were mutated to recognize TAG codon and insert m-Cl-Tyr in its place (Mj-TyrRS and Mj-tRNATyr, Fig 1A) (Sakamoto et al.,

2009). The amounts of expressed pore forming proteins with m-Cl-Tyr were assessed using western blot, where anti-His antibodies were used to identify the target protein (Fig 1B).

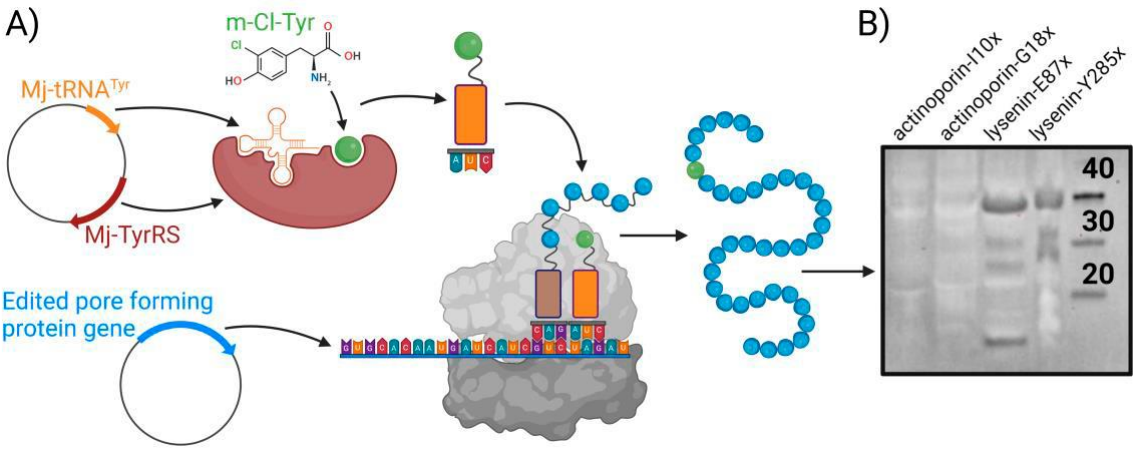

Figure 1: A) The preparation of nanopore monomers (blue) with m-Cl-Tyr (green) using orthogonal TyrRS (red) and tRNATyr (orange) from M. jannaschii that have been modified to recognize the ncAA and the TAG stop codon. B) Western blot analysis of E. coli lysates expressing mutant actinoporin and lysenin containing m-Cl-Tyr. On the right is the molecular weight marker, the mass in kDa is indicated above each band. Under the conditions tested, the expression of two actinoporin mutants was unsuccessful, while the bands at 40 kDa in the other two samples represent successfully expressed lysenin mutants. The low molecular weight bands at the bottom of the gel correspond to the truncated protein in which translation was terminated by RF1 instead of ncAA insertion.

DISCUSSION

The yields of the prepared protein containing the ncAA can be further improved by engineering a more catalytically efficient aaRS•tRNA pair (Vargas-Rodriguez et al., 2018) or by employing an E. coli strain in which the release factor 1 (RF1) is deleted and all of its TAG codons were substituted with another stop codon. In that case, RF1 would not compete with orthogonal tRNA for TAG codon binding allowing successful read-through of the internal stop codon(s) (Isaacs et al., 2011). In the future, we will also prepare proteins containing SF5-Phe, assess the pore-forming activity of the ncAA-containing proteins by hemolysis assays, and verify the presence of ncAAs in expressed proteins by mass spectrometry. The enhanced stability will be examined by circular dichroism to determine their usefulness in nanopore biosensing under denaturing conditions.

CONCLUSIONS

With the use of genetic code expansion, a large variety of ncAAs can be introduced into proteins diversifying and enhancing their use in numerous applications.

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CERAMIC-BASED CARRIERS AS A BIOFILMS INTERFACE: DESIGN AND MEDICAL APPLICATIONS

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Hydrogels, recognized for their unique three-dimensional polymer and water compositions, hold significant potential in the biomedical sector, despite their inherent mechanical limitations. Consequently, the development of hydrogel-based biomaterials with adjustable mechanical properties, cell-binding capabilities, and intricate structures has become a potent strategy for managing cell attachment and growth in tissue engineering [1]. In our research, we incorporated bioceramic colloidal micro/nanoparticles as a essential element of this system. We experimented with particles of varying Ca2+/Mg2+ ratios and sizes (1 to 8 µm), combining them with a gellan gum (GG) solution to observe the spontaneous formation of hydrogel-particle composites [2]. These particles serve several purposes:

1) effectively crosslink with GG, triggering hydrogel formation via the release of divalent cations known to bind with GG polymer chains;

2) enhanced the mechanical strength of the hydrogel;

3) they function as a delivery system, with loading efficiency and capacity contingent on particle size, enzyme loading duration, and variations in container compositions and enzyme concentrations;

4) the samples that most effectively stimulated cell growth were found to contain calcium carbonate and hydroxymagnesite, which boosted cell proliferation and hydroxyapatite formation.

By refining the synthesis and particle modification process, we were able to prepare an enzyme delivery system with functionalized protective layers, as well as a diagnostic platform capable of providing a surface-enhanced Raman scattering effect (SERS). Our findings indicate that particle size impacts their morphology, which subsequently influences the activity of the encapsulated enzymes. Given the therapeutic effect on osteoblastic cells, relatively high loading capacity, biocompatibility, and ease of production, the carriers we developed show promise for effective drug delivery, particularly in bone reconstruction applications.

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Jožef Stefan Institute

SURFBIO

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The Laboratory for Colloid Biology is working on the new discipline focused on the study of the interaction between different microbial cells and between cells and surfaces. The research in this field might give scientific community a completely new tool for manipulation of structures of bacterial communities.

From the Jožef Stefan Institute, Sloveniaś leading scientific research institute, together with five top research & innovation partners across Europe, SURFBIO project partners are creating an Innovation Hub to study micro-be–surface by using high-tech methodologies and equipment

SURFBIO project aim to provide researchers, academic institutions, industry and policy makers with training services and assessments to optimise novel materials for a variety of applications. Understanding the interactions of the colloids (microorganisms and biomolecules) with surfaces and between themselves is a key factor that can lead to improvements in several fields, such as the biotechnology industry or the development of nano-carriers for drug delivery. These interactions can be studied and analysed by applying different tools and techniques, and this is the SURFBIO main objective.

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