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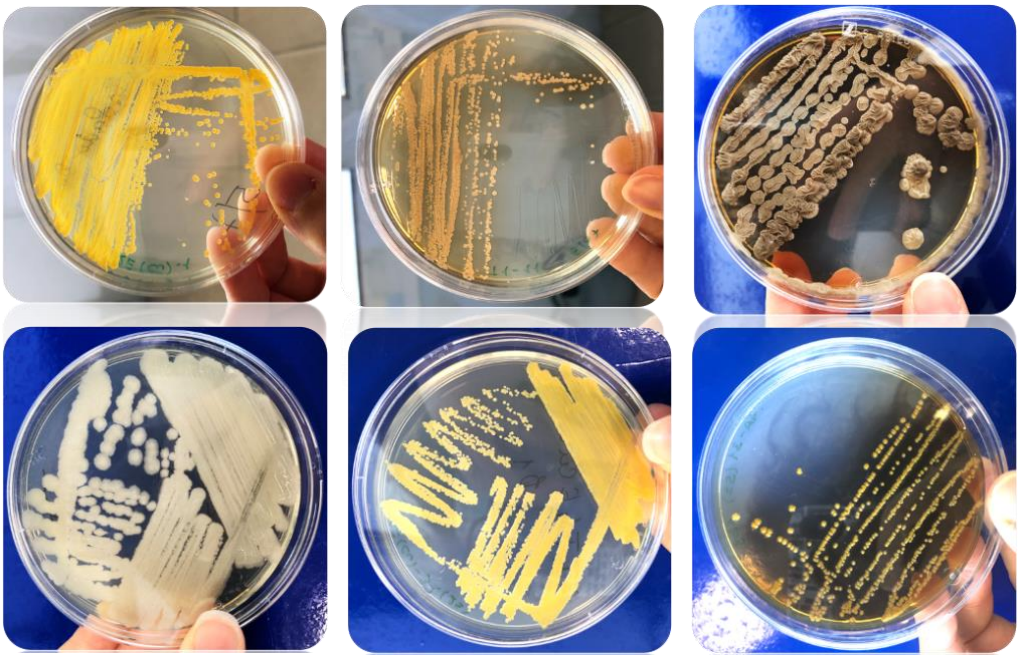
Phy2SUDOE

European Regional Development Fund

RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE



PRODUCT 3.3

COLLECTION OF MICROBIAL STRAINS WITH BIOTECHNOLOGICAL POTENTIAL

GT3 - Identification and Conservation of endemic biodiversity of contaminated sites for potential exploitation in biotechnological applications

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"*Advancing in the application of innovative phytomanagement strategies in contaminated areas of the SUDOE space*"

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Authors: Helena Moreira (UCP-CRP), Sofia Pereira (UCP-CRP), Ana Sofia Sousa (UCP-CRP), Lur Epelde (NEIKER), Mikel Anza (NEIKER), Beatriz Rodríguez-Garrido (MBG-CSIC), Carmela Monterroso-Martínez (USC), Ángeles Prieto-Fernández (MBG-CSIC), Carmen Trasar-Cepeda (MBG-CSIC), Michel Mench (INRA), José María Becerril (UPV/EHU), Paula Castro (UCP-CRP), Manu Soto (UPV/EHU), Lur Epelde (NEIKER), Álvaro Nunes de Sousa (CloverStrategy), Juan Vilela (CEA), Carlos Garbisu (NEIKER)

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1. INTRODUCTION

Mining areas are known to harbor a diverse array of microbial strains with significant biotechnological potential. These environments exhibit extreme conditions, including high concentrations of metals and metalloids, acidic/alkaline pH, and limited nutrient availability. Within these challenging settings, microbial communities have adapted and developed specialized adaptations and capabilities. Therefore, these microorganisms can offer valuable opportunities for exploration and utilization. For example, certain strains have demonstrated the ability to accumulate or precipitate metal(loid)s, making them valuable for the remediation of metal(loid)-contaminated environments or the recovery of valuable metals from mine wastes. Additionally, some microorganisms possess the capacity to produce enzymes that can degrade organic compounds, while others exhibit plant growth-promoting traits, making them suitable for bioaugmentation remediation strategies. Moreover, the study of the genomes and metabolic pathways of microorganisms from mining areas can provide insights into novel mechanisms of metal resistance, metal transformation, and other biotechnologically relevant traits. This knowledge can drive advancements in biotechnology and contribute to the development of sustainable solutions, e.g., mining-related challenges and environmental remediation. The exploration and study of microbial communities in mining areas can also uncover new species.

Through prospecting and screening efforts, microorganisms, namely bacteria, from mines have the potential to contribute to the development of environmental remediation and the production of valuable biotechnological products. Therefore, bacterial strains, from the mines of Sentein-Bulard, La Plagne (FR), Borralha (PT), and Lanestosa (ES), were isolated and characterized according to e.g., their metal(loid) tolerance and plant growth-promoting traits (Deliverable 3.3.1) and are now preserved at Escola Superior de Biotecnologia da Universidade Católica Portuguesa (Porto, Portugal) and in NEIKER (Bilbao, Spain). Each strain within the collection is identified, and stored under specific conditions to ensure its viability and stability over time. This repository is maintained especially for research and industrial purposes.

2. IMPORTANCE OF THE MICROBIAL COLLECTION

The microbial collection done under the project can play a crucial role in advancing scientific research, biotechnological innovation, and environmental restoration. The collection is a repository of diverse and well-characterized microorganisms and can provide a vast resource of biological material to be used in a wide range of applications, which can include:

- Environmental remediation: microbial strains that exhibit desirable traits, such as metal tolerance, plant growth-promoting traits, metal(loid) accumulation, and/or metabolic pathways that can contribute to pollutant degradation can be selected for bioaugmentation approaches for environmental remediation.
- Development of sustainable solutions: microbial strains with biotechnological potential can contribute to the development of sustainable solutions in various industries. For example, they can be used to produce enzymes for industrial processes, which can replace traditional chemical catalysts and reduce the environmental impact. Microbes can also be engineered to degrade pollutants or produce biofuels, offering eco-friendly alternatives to conventional practices.
- Understanding microbial diversity and evolution: building a collection of microbial strains allows researchers to study and understand the vast diversity of microorganisms. By exploring their genomes, metabolic pathways, and interactions, scientists can gain insights into microbial evolution, adaptation, and ecology.
- Biotechnological innovation and commercialization: The collection of microbial strains serves as a valuable resource for biotechnological innovation and commercialization. Companies and research institutions can access this collection to identify microorganisms suitable for industrial processes, develop biotechnological products, and explore new applications.
- Conservation of microbial diversity: many microorganisms are threatened by habitat loss. Building a collection of microbial strains to the conservation of microbial diversity. By preserving and studying these microorganisms, we can better understand their ecological roles.
- Discovery of novel bioactive compounds: bacteria are a rich source of bioactive compounds with various applications in biotechnology, such as the production of enzymes, antibiotics, biofuels, and bioplastics.

Overall, building a collection of microbial strains with biotechnological potential is essential for advancing scientific knowledge, developing sustainable solutions for the remediation of contaminated sites, promoting innovation, and conserving microbial diversity, ultimately benefiting various fields and contributing to the advancement of biotechnology.

3. USE OF THE MICROBIAL COLLECTION FOR BIOAUGMENTATION

Bioaugmentation is a biotechnological approach that involves the introduction of specific microbial strains or consortia into an environment to enhance natural biological processes, promote plant growth and tolerance to contaminants, or influence the

accumulation of trace elements and/or the degradation of organic contaminants. In bioaugmentation, carefully selected microbial strains with specialized metabolic capabilities are introduced to target contaminated sites where indigenous microbial populations may be insufficient or ineffective. These introduced strains possess unique enzymatic activities or genetic traits that enable them to efficiently degrade/contain a wide range of contaminants and/or influence plant growth. The success of bioaugmentation relies on factors such as strain selection, compatibility with the target environment, proper delivery methods, inocula size, and ecological interactions between the introduced strains and the existing microbial community.

Based on their origin, microorganisms utilized as bioinoculants can be categorized as follows:

- Autochthonous: these microorganisms are sourced from the specific site earmarked for phytomanagement. Initially, they are collected from the site, cultivated *ex-situ*, and subsequently reintroduced into the soil.
- Allochthonous: allochthonous microorganisms are gathered from contaminated areas different from the target site for phytomanagement. These microorganisms are then cultivated *ex-situ* and later inoculated into the soil.

Another approach involves using commercial products. However, the effectiveness of these products in achieving desired outcomes can be limited if their composition lacks strains that are well-adapted to high concentrations of hazardous metal(loid)s or do not have the necessary plant growth-promoting traits.

The use of the strains of the microbial collection for bioaugmentation approaches in mining sites can offer promising results. These strains (plant growth-promoting bacteria - PGPB) are metal(loid) tolerant and have plant growth-promoting traits (Fig. 1) that can help, e.g., the revegetation of contaminated sites, by improving the plant growth and nutrient acquisition.

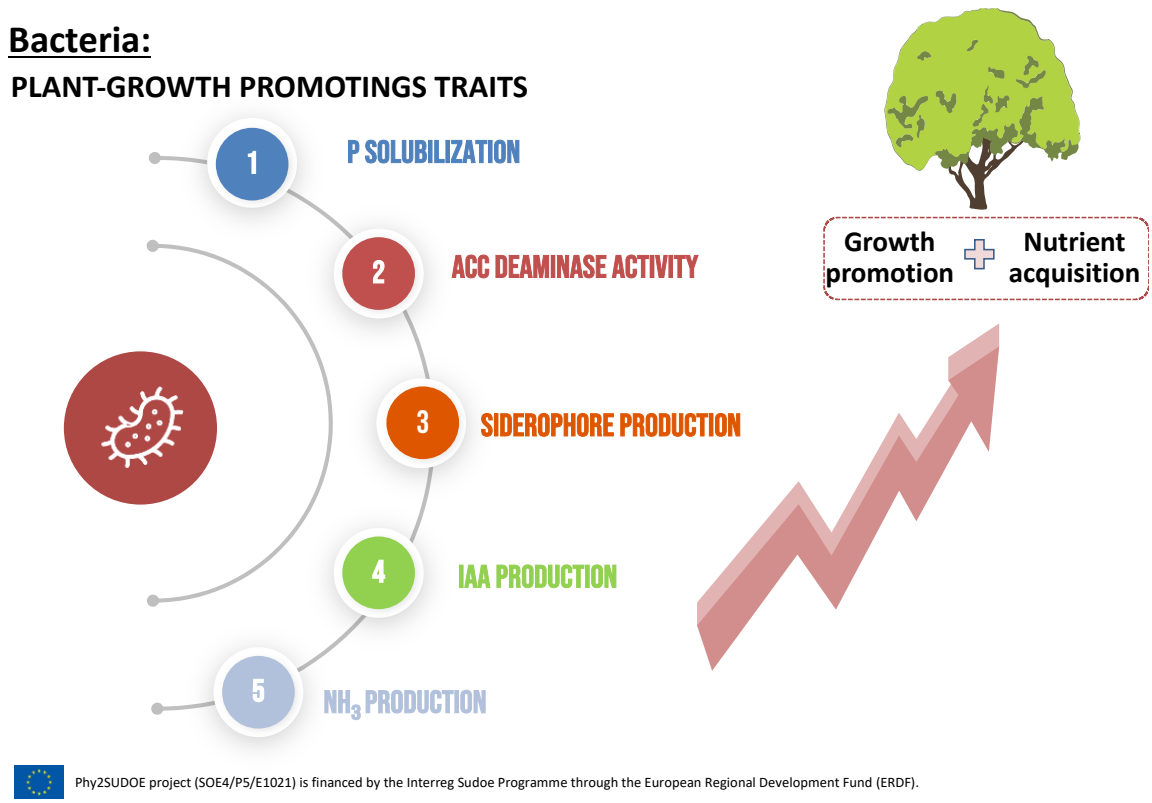

Figure1. Overview of the effect on plants of bacterial growth-promoting traits.

Metal-resistant PGPBs have been reported to employ several mechanisms involved in plant establishment in contaminated areas, as follows:

- **Phosphorous solubilization:** PGPB can solubilize phosphorus ($H_2PO_4^-$ and HPO_4^{2-}) to make it available for absorption by plant roots, as phosphorus is often a limiting nutrient for growth enhancement. This process involves acidification, chelation, exchange reactions, and the release of organic acids or the mineralization of organic phosphates through the release of extracellular phosphatases.
- **Production of phytohormones:** PGPB can synthesize auxins, such as indole-3-acetic acid (IAA), cytokinins, and gibberellins, which promote germination, plant growth, and the reinforcement of the plant defense system.
- **Production of siderophores:** In response to low iron levels in the rhizosphere, PGPB produces siderophores, which act as soluble Fe^{3+} binding agents, sequestering iron from the soil and providing it to plants. By reducing iron availability, they also compromise the survival of pathogens. The presence of

metals like Cd and Zn can also stimulate siderophore production, which can also favor metal chelation.

- **Decrease of ethylene levels:** ethylene hormone is overproduced under stress conditions and inhibits plant growth, promotes senescence and abscission, or causes seedling death. PGPB can lower ethylene levels by synthesizing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyzes ACC (a precursor of ethylene) to α-ketobutyrate and ammonia, which can serve as a sole nitrogen source.
- **Nitrogen fixation:** PGPB enhances nitrogen sufficiency through symbiotic and non-symbiotic fixation. In symbiotic fixation, bacteria fix atmospheric nitrogen in specialized nodule structures, while in non-symbiotic fixation, non-symbiotic nitrogen-fixers fix nitrogen in the soil.
- **Metal immobilization/mobilization:** bacteria have a high surface area-to-volume ratio, allowing them to decrease metal toxicity through bioaccumulation and biosorption mechanisms.
- **Induction of systemic resistance in plants:** plant stress triggers the activation of their defense systems to protect against oxidative damage, and PGPB inoculation can enhance this response.
- **Protection against diseases:** PGPB can produce enzymes such as chitinases, proteases, or lipases that induce the breakdown of fungal pathogens' cell walls.

Research has revealed additional potential benefits of PGPB beyond enhancing plant growth, particularly in alleviating biotic and abiotic stress in plants. These findings have led to the utilization of PGPB in promoting the growth of plants exposed to various stressors such as drought, organic contaminants, salinity, phytopathogens, and metal(loid)s. The beneficial effects of PGPB are particularly evident under sub-optimal conditions for host plants, as these are the situations where PGPB exerts the most significant influence. In addition, PGPB benefit plants through multiple mechanisms, which can act simultaneously or sequentially during different stages of the host plants' life cycle.

3. COLLECTIONS OF BACTERIAL STRAINS

The collections of bacterial strains (Tables 1 and 2) are stored at Escola Superior de Biotecnologia da Universidade Católica Portuguesa (Porto, Portugal) and at NEIKER (Bilbao, Spain), whose responsible persons are, respectively, Prof. Paula Castro and Dr. Mikel Anza.

Table 1. Collection of Bacterial Strains of Escola Superior de Biotecnologia (UCP-CRP) collected from the Borralha Mine.

Nº Strain	Assigned taxon	SID	PHO	IAA	NH_3	SURF	BIOF
1	*Rhodococcus boritolerans*	+	+	44.4	+	0	0.10
2	*Burkholderia diffusa*	++	3.0 ± 0.7	86.6	++	0	0.57
3	*Acinetobacter courvalinii*	+	3.2 ± 0.2	65.5	+	+	1.21
4	*Pseudomonas glycinae*	+	+	127.8	+++++		
5	*Bacillus wiedmanni*	++	0	44.8	++	++	0.07
6	*Bacillus atrophaeus*	+++++	0	25.4	++	++	
7	*Priestia megaterium*	+	+	36.9	+	0	1.34
8	*Bacillus* sp.	+	+	17.4	++	0	0.68
9	*Bacillus licheniformis*	+++++	+	30.2	++	0	
10	*Rhodococcus opacus*	++	+	82.4	++	0	0.34
11	*Nocardia* sp.	++	0	19.7	+	0	
12	*Kitasatospora aureofaciens*	+++++	+	38.7	++	+	0.83
13	*Paenarthrobacter nicotinovorans*	+	0	49.0	+	+	0.16
14	*Bacillus* sp.	+	+	22.6	+	+	0.49
15	*Arthrobacter sp.*	++	+	65.2	+	0	0.31
16	*Bacillus paramycoides*	+	0	29.7	++	++	
17	*Streptomyces mauvecolor*	++++	0	33.1	+	0	0.02
18	*Bacillus* sp.	+	0	62.2	+	0	0.63
19	*Amycolatopsis* sp.	+++	0	32.0	++	0	
20	*Arthrobacter* sp.	+	+	304.9	+	+	1.46
21	*Burkholderia* sp.	+++	3.8 ± 0.58	45.1	+++	0	3.00
22	*Paenarthrobacter nitroguajacolicus*	+	0	86.0	+	0	0.45
23	*Acinetobacter calcoaceticus*	+	0	30.6	+	0	1.63
24	*Bacillus muralis*	+	0	60.9	+	0	2.50

25	*Bacillus nakamurai*	++++	0	33.3	++	++	3.79
26	*Bacillus thuringiensis*	0	0	43.5	++	++	1.63
27	*Novosphingobium resinovorum*	++	0	64.2	++	0	0.83
28	*Streptomyces* sp.	+++++	+	56.3	++	+	1.67
29	*Bacillus* sp.	+	0	46.4	++++	++	
30	*Bacillus* sp.	++++	0	25.4	++++	++	2.74
31	*Burkholderia* sp.	++	0	85.3	+++++	0	1.37
32	*Paenibacillus* sp.	++	0	20.2	+	+	0.77
33	*Burkholderia contaminans*	+	3.8 ±0.51	102.5	0		
34	*Lelliota amnigena*	0	+	71.9	++		
35	*Cupriavidus pauculus*	0	0	119.7	+		
36	*Bacillus wiedmanni*	0	0	33.4	++		

Table 2. Collection of Bacterial Strains of NEIKER, collected from Lanestosa (SP), Sentein Mine (FR) and La Plagne (FR) mines.

Nº Strain	Assigned taxon	SID	PHO	IAA	ACC	SURF	BIOF
1	*Pseudomonas protegens*	XXX	XXX PLUS	3.85		XXX	
2	*Pseudomonas helmanticensis*	XXX PLUS	XXX	2.06		0	
3	*Herminiimonas contaminans*			34.90		XX	
4	*Variovorax boronicumulans*	XX		19.98		0	
5	*Variovorax boronicumulans*	X	X	6.34	6.00	0	
6	*Variovorax boronicumulans*			18.05	4.94	0	
7	*Pseudomonas koreensis*	XXX	XXX	1.46		0	
8	*Rhodococcus gingshengii*	XXX PLUS	X	0.58		0	
9	*Variovorax boronicumulans*			11.46	13.06	0	
10	*Pseudomonas helmanticensis*	XXX	X	8.48		0	
11	*Lentzea albidocapillata*	XX	X	0.15		XXX	
12	*Paenarthrobacter nitroguajacolicus*	XXX	XX	0.08		0	
13	*Streptomyces cirratus*	XXX PLUS	X	0.02		0	
14	*Staphylococcus saprophyticus*	XXX	X	0.04		X	
15	*Pseudomonas asplenii*	XX	XX	0.05		0	0.31
16	*Rhodococcus fascians*	X	X	0.09	30.44	0	

17	*Variovorax boronicumulans*	XX		0.22	3.92	0	0.02
18	*Nocardia coeliaca*	XXX	XX	0.09		0	0.63
19	*Serratia plymuthica*	XXX	X	0.20		0	
20	*Cupriavidus basilensis*	X		0.04	10.45	0	1.46
21	*Pseudomonas protegens*	XXX	XXXPLUS	0.05		0	3.00
22	*Variovorax boronicumulans*	XX	X	0.10	7.38	0	0.45

The description of the sites can be found at https://www.phytosudoe.eu/en/.

4. DISSEMINATION OF MICROBIAL COLLECTION

The dissemination of the bacterial collection will be done through various channels to reach a wide audience, as follows:

- Website: a dedicated webpage on the research centers websites specifically for the bacterial collection will be created. It will be provided detailed information about the collection, its purpose, available strains, and access procedures.
- Collaborations and Networking: collaborations with other universities, research institutions, and industry partners will be engaged, and connections with researchers and scientists working in relevant fields will be established. Conferences, workshops, and scientific meetings will be used to present the collection, share research findings, and build collaborations.
- Publications and Research Articles: the bacterial collection will be used for research purposes and the findings will be published in scientific journals. This will help to showcase the collection's scientific value and promote its usage among the scientific community.
- Outreach and Public Engagement: outreach activities will be used to engage the general public and raise awareness about the importance of microbial collections.
- Social Media and Newsletters: social media platforms, namely Facebook, or LinkedIn, will be used to highlight the bacterial collection. New strains, publications, events, and other relevant information will be regularly posted.
- Collaboration with Industry: collaborations with industries or companies that can benefit from the bacterial collection will be pursued.

Proper protocols and permissions for sharing and distributing microbial strains, and adhering to ethical and legal guidelines will also be outlined.

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