Role of bacteria and mycorrhizal fungi in phytomining: status and future perspectives

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The ever-growing demand for metals has been fueling the development of alternative technologies to recover elements of interest from sub-economic deposits. One such example is phytomining, a technique that employs plants with the ability to accumulate astonishing amounts of metals in their tissues. These plants, called hyperaccumulators, can be used to retrieve metals such as Ni or Au from ultramafic soils and mine tailings, respectively. The rhizosphere of hyperaccumulators is often populated by large amounts of microorganisms mainly consisting of bacteria and mycorrhizal fungi. These microorganisms can enhance plant biomass production, reduce metal phytotoxicity, and increase the bioavailability of certain elements of interest. In this chapter we review the role of bacteria and mycorrhizal fungi in phytomining, and outline future perspectives of their application to enhance this green metal-recovery technique.

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1. Introduction

Population growth and the expectancy of improved living standards leads to the rising demand of chemical elements, since they are fundamentally linked to industrial development (Whiting et al., 2002). Mine sites are the primary source for recovering valuable metals suited for applications, such as clean energy technologies and medicinal industry. However, conventional methods of extraction take a negative toll on the environment, producing large amounts of mine waste, causing water and soil contamination, destruction of habitats and loss of biodiversity (Thijs et al., 2017). Commercial mining also requires large capital investments and the existence of easily accessible ores that possess a high concentration of target metals. Ore bodies of this nature only occur in small localized areas and are declining due to its disarrayed extraction (Sheoran et al., 2009). The issues associated with conventional mining and the need of a global shift towards sustainable development have prompted the advance of phytomining during the last three decades. Phytomining is an alternative technology based on the use of certain plants to recover valuable metals from mineralized or polluted soils (Brooks et al., 1998). This innovative, solar driven, environment friendly and low-cost approach, offers the possibility of exploiting resources from low-grade ores, which are subeconomic for conventional mining. Moreover, phytomining is virtually harmless to the environment, promotes its remediation, and is aesthetically pleasing, thus having high probability of public acceptance (Nedelkoska and Doran, 2000; Novo et al., 2017).

Plants used for phytomining can accumulate astonishing amounts of metals, such as nickel (Ni), cobalt (Co), thallium (Tl) and rhenium (Re), in their shoots without showing significant signs of toxicity (Novo et al., 2018; van der Ent et al., 2013). These plants, called hyperaccumulators, constitute an example of the curious and outstanding adaptations of flora to metalliferous environments, which are adverse to normal plants (Whiting et al., 2002). Species can be classified as hyperaccumulators if their shoot concentrations surpass the thresholds defined for each element (van der Ent et al., 2013). Nickel hyperaccumulators, for instance, are defined as plants that accumulate Ni in their dry leaf tissues to concentrations surpassing 1000 mg kg\(^{-1}\), i.e., 0.1% of their leaf dry mass (Brooks et al., 1977). The basic mechanisms governing hyperaccumulation are: a high rate of metal uptake from the soil, an efficient root to shoot translocation, and a great ability to detoxify and sequester metals in leaves (Rascio and Navari-Izzo, 2011).

The phytomining process consists in the cultivation of hyperaccumulators (preferably native or naturalized non-invasive species) in selected metal-rich substrates, followed by the harvest of the aboveground parts and transformation of the biomass to obtain valuable end products (Kidd et al., 2018; Novo et al., 2017). There are two main approaches to be considered for phytomining: (i) phytomining on degraded mine soils and quarries, as part of a simultaneous rehabilitation strategy (along with
phytoremediation), where the revenue obtained through phytomining finances its costs; (ii) phytomining in natural metal rich soils unsuitable for conventional agriculture and grazing (e.g. serpentine soils) as part of an integrated agricultural chain known as agromining (van der Ent et al., 2015). For this phytotechnology to be economically profitable, high plant biomass yield and elevated metal concentrations in aerial plant parts are required, since these aspects govern the quantity of metal to be harvested from each plant (harvestable mount) (Novo et al., 2015). Nickel phytomining is particularly promising due to the high market price of this element, the great variety of Ni hyperaccumulator species (which comprise nearly 90% of the reported hyperaccumulators), and the abundance of Ni rich ultramafic soils worldwide (van der Ent et al., 2015). Furthermore, there are numerous studies on Ni phytomining showing its great economic potential (Nkrumah et al., 2016, 2019; Novo et al., 2017). Nonetheless, there are limitations to the application of this phytotechnology due to slow growth of hyperaccumulators, low biomass production, shallow root systems, and different factors influencing metal uptake such as the solubility and availability of elements in soil (Ghosh and Singh, 2005). Thus, it is imperative the study of agronomical practices for optimization of phytomining systems, which may lead to the improvement of metal extraction, and thereby to greater economic profits.

Microbial-assisted phytomining is a relatively recent concept that consists in the incorporation of microbial inoculants into phytomining systems, for overcoming limitations in this process. The rhizosphere of all plants, including hyperaccumulators, is inhabited by a high diversity of microorganisms, some of which are shown to have the ability for assisting plant growth and regulating metal uptake (Thijs et al., 2017). Microorganisms play a very important part in nutrient uptake and transport, attenuate stress and protect the plant from pathogens (Lugtenberg and Kamilova, 2009; Rashid et al., 2016). Serpentine soil microbes, such as bacteria and fungi, are considered important drivers of ecosystem diversity, often featuring critical roles in soil nutrient cycling and stimulating plant growth. It should be noted that serpentinic environments are poor in nutrients essential to plants [such as nitrogen (N), phosphorus (P) and potassium], and organic matter, and rich in metals (such as Ni, Co and chromium) (Alves et al., 2019; Benizri and Kidd, 2018; Kidd et al., 2018). Microorganisms can also contribute for stress alleviation in plants, metal tolerance, mobilization and uptake (Thijs et al., 2017).

The characterization and study of the rhizosphere microbiome of Ni hyperaccumulators is crucial for assessing endemic soil biological and ecological friendly phytomining techniques (Sheoran et al., 2016; Thijs et al., 2017). Plant-microbe interactions play an important role in plant biomass yield and metal accumulation, and therefore, the application of inoculants into phytomining systems may overcome several limitations in this process. In addition, the use of inoculants can minimize the application of other agronomic optimizing agents, such as inorganic fertilizers, organic
compounds, phytohormones and chelating substances, and consequently reduce the corresponding costs (Álvarez-López et al., 2016; Thijs et al., 2017). This chapter overviews the status of microbial-assisted phytomining, focusing on the roles of plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi, and their potential to enhance Ni phytomining.

### 2. Rhizosphere and its microorganisms

The rhizosphere is defined as the volume of soil adjacent to living plant roots that is physically and chemically influenced by root activity and growth (Benizri and Kidd, 2018; Nguyen, 2003). It constitutes a very dynamic microsystem in which roots, microorganisms and soil interact (Thijs et al., 2017). There is a high microbial diversity and activity in the rhizosphere that contrasts with the microbial communities inhabiting non-rhizosphere or bulk soil (Baudoin et al., 2003). This is a result of the overall plant-rhizosphere microbes interaction and of several biotic and abiotic factors, such as root exudation, inorganic nutrients, competition for iron (Fe) and carbon (C), soil pH and temperature (Baudoin et al., 2003; Benizri and Kidd, 2018; Khan, 2005). Root exudates are especially important for rhizosphere microorganisms because they represent a source of C (and possibly N) and energy, driving microbial activity, diversity and growth (Bowen and Rovira, 1999). Thereby, compared to bulk soil the rhizosphere is characterized by higher concentrations of nutrients and labile organic C, representing a unique “hot spot” in terms of microbial ecology (Hinsinger et al., 2006). It was estimated that the rhizosphere contains up to $10^{11}$ microbial cells per gram soil, from thousands of different species, comprising bacteria, fungi and archaea (Mendes et al., 2013). The important flux of C in the rhizosphere results of large amounts of this element that is transferred to the root and lost through rhizodeposition in the soil (Nguyen, 2003). There are five types of rhizodeposits: secretions, diffusates, lysates, gases and mucilage (Nguyen, 2003). Rhizodeposits serve as a nutrient source and are key determinants of rhizosphere microbial community structure, since the type and composition of secretions are responsible for altering soil microbial diversity (Chaparro et al., 2012). Plants provide vitamins, hormones, toxins and antagonistic compounds that have a selective effect on microorganisms, favoring species beneficial to plants, that promote health and productivity (Benizri and Kidd, 2018; Warembourg, 1997). Thus, while plants support microbial growth, bacteria and fungi promote plant development by improving nutrient uptake, attenuating abiotic stress and protecting the plant from pathogens (Lugtenberg and Kamilova, 2009; Rashid et al., 2016). Figure 1 displays the main benefits of plant growth-promoting microorganisms.

Rhizosphere microorganism drive the cycle of important elements (such as C, N and P) through their enzyme activity, affecting soil structure and functions (Benizri and Kidd, 2018). They are often responsible for increasing the availability of important plant
macronutrients and the extent of the root system, leading, in both cases, to the improvement of nutrient uptake by plants. Rhizosphere beneficial microbiome may also influence plant-pathogen interactions and plant growth, as previously mentioned, by several mechanisms including the production of hormones and antibiotics (Benizri and Kidd, 2018).

Figure 1 – Main functions of plant beneficial rhizosphere microorganisms.

2.1. Rhizosphere microbiome of hyperaccumulator plants

Despite the occurrence of high concentrations of metals in soils, the rhizosphere of hyperaccumulator plants is inhabited by a large amount of plant beneficial microorganisms (Abou-Shanab et al., 2003). These microorganisms are responsible for assisting plants in tolerating high metal toxicity (Manoj et al., 2020). Natural metal rich sites, like ultramafic soils, constitute an abiotic factor that modulates microbial activity in the rhizosphere (Kidd et al., 2018). Besides, it is recorded that Ni hyperaccumulator species select metal tolerant microbial strains that are linked to metal hyperaccumulation, with some unique microbial groups detected in large quantities at these metal rich sites (Abou-Shanab et al., 2003; Mengoni et al., 2001; Thijs et al., 2017).

The beneficial effects of rhizosphere microbiome on plant growth, functions, physiology and efficiency in metal accumulation, have made the study of these microorganisms very attractive during the last couple of decades (Ma et al., 2011; Weyens et al., 2009). However, there is still little information about the diversity and
functions of microbial communities in metal rich sites (Khan, 2005). Microorganisms can have a large effect on phytoremediation and phytomining, and therefore, have the potential to be used as inoculants to improve the efficiency of these phytotechnologies. Reports show that PGPR and fungal symbionts contribute for plant growth and metal tolerance in ultramafic environments and enhance Ni bioavailability and accumulation by hyperaccumulator plants (Idris et al., 2004; Kidd et al., 2018).

3. Plant growth-promoting rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) are plant associated, free-living, soil-borne bacteria, that are responsible for enhancing plant growth (Manoj et al., 2020). In order to classify as PGPR, bacteria must be root colonizers and capable to survive, multiply, adapt and compete with other microorganisms until expressing plant growth promotion (Kloeper, 1994). Plant growth-promoting rhizobacteria comprise a great number of bacterial genera that can be categorized according to their functional properties as: i) biofertilizers (increase availability of nutrients to the plant); ii) phytostimulators (promote plant growth through the production of hormones); iii) rhizoremediators (break down organic contaminants and modulate metal solubilization); and iv) biopesticides (control diseases and plant pathogens by synthetizing antibiotics and antifungal metabolites) (Somers et al., 2004). Plant growth-promoting rhizobacteria may also have the capacity to increase metal availability and accumulation by producing compounds such as siderophores, organic acids and biosurfactants (Sessitsch et al., 2013). The compounds can acidify the rhizosphere, decrease soil pH, generate oxidation-reduction reactions and alter the mobility of metals through chelation, precipitation and immobilization (Sessitsch et al., 2013). Microbial siderophores are considered the major metal chelating agents in metal solubilization (Ahemad, 2014).

Plant growth-promoting rhizobacteria can influence plant growth through direct and indirect mechanisms (Table 1). The former consists in the promotion of plant growth by facilitating nutrient uptake or via synthesis of phytohormones (Novo et al., 2018). Bacteria strains can stimulate root growth, cell division and elongation and boost metabolic stabilization (Manoj et al., 2020). Indirect mechanisms are responsible for reducing or suppressing plant pathogens by producing antagonistic compounds, improving induction of systemic resistance and suppressing metal toxicity in the case of plants that inhabit metal rich soils (Saraf et al., 2014). Indirect mechanisms are not involved directly in plant growth but play a role in reducing plant stress caused by biotic (e.g. pathogenic attacks) and abiotic (e.g. heavy metal toxicity) factors (Manoj et al., 2020). Plant growth-promoting rhizobacteria direct mechanisms consist in N fixation, phosphate solubilization, Fe sequestration and in the production of phytohormones (such as auxins, cytokinins and gibberellines) and modulation. Indirect mechanisms
comprise biocontrol via the production of compounds known as allelochemicals (such as antibiotics, lytic enzymes and siderophores) and in eliciting induced systemic resistance (ISR) (Glick, 2012; Novo et al., 2018).

<table>
<thead>
<tr>
<th>Mechanism type</th>
<th>Function</th>
<th>Synthesized compound</th>
</tr>
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<tbody>
<tr>
<td>Direct</td>
<td>Nitrogen Fixation</td>
<td>Nitrogenase</td>
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<tr>
<td></td>
<td>Phosphate Solubilization</td>
<td>Low Molecular Weight Organic Acids, Phosphatases, C-P Lyases</td>
</tr>
<tr>
<td></td>
<td>Iron Sequestration</td>
<td>Siderophores</td>
</tr>
<tr>
<td></td>
<td>Phytohormones Production and Modulation</td>
<td>Auxins, Cytokins, Gibberellins, Ethylene</td>
</tr>
<tr>
<td>Indirect</td>
<td>Biocontrol</td>
<td>Antibiotics, Lytic Enzymes, Siderophores</td>
</tr>
</tbody>
</table>

### 3.1. Nitrogen fixation, phosphate solubilization and iron sequestration

Plant growth-promoting rhizobacteria can increase availability of nutrients that are important to plants such as, N, phosphate and Fe. Nitrogen has an important role in plant growth and development; however, plants are unable to utilize atmospheric N₂ directly (Novo et al., 2018). Nitrogen-fixing bacteria are responsible for continuously converting atmospheric N₂ into plant available forms such as ammonia or nitrate. This process is carried out by a complex enzyme system called nitrogenase (Hoffman et al., 2014). Phosphorus also constitutes a very important macronutrient for plants and although it is relatively abundant, it is generally in an insoluble form, not available to plants (Glick, 2012). Bacteria play an important role in P solubilization, supplying bioavailable P to plants (Novo et al., 2018). This process occurs through the secretion of enzymes (such as phosphatases and C-P lyases) and low molecular weight organic acids (such as acetic, citric, lactic and gluconic acid) (Goswami et al., 2016). Some PGPR are able to produce low molecular weight compounds known as siderophores (Novo et al., 2018). Siderophores are proteins that exhibit a very high affinity for Fe³⁺ (Rajkumar et al., 2010). They are responsible for converting Fe³⁺, which is unavailable for plants, into Fe²⁺ that is available for plant uptake and required for several metabolic activities (Manoj et al., 2020). Siderophores are also able to bind to a wide range of metals (besides Fe), thus enhancing their availability for plant uptake, and potentially improving metal hyperaccumulation (Sheoran et al., 2016).

### 3.2. Phytohormone production and modulation

Plant growth-promoting rhizobacteria can provide plants with a variety of phytohormones including, auxins, cytokinins and gibberellins, and produce enzymes that reduce ethylene levels (Goswami et al., 2016; Manoj et al., 2020). These substances
enhance plant growth, even at low concentrations, by modulating several physiological and metabolic functions of the plant under metal stress (Manoj et al., 2020). Phytohormones synthetized by PGPR may prevent detrimental effects of metals in plants inhabiting metal rich sites, and consequently increase metal accumulation (Goswami et al., 2016; Ma et al., 2016b).

Auxins possess a valuable role in root system development and PGPR synthetize several forms of auxins, with the most important being indole-3-acetic acid (IAA) (Manoj et al., 2020). This auxin is responsible for stimulating root growth by cell division and tissue expansion, and stimulate seed and tuber germination, among other beneficial effects (Goswami et al., 2016; Novo et al., 2018). Cytokinins are the major bacterial phytohormones after auxins and can improve plant growth by, for instance, eliciting cell division and differentiation and enhancing leaf expansion (Bhattacharyya and Jha, 2012). Gibberellins are able to promote shoot growth by cell elongation, division and differentiation (Wang et al., 2015). Lastly, PGPR are also responsible for producing an enzyme called 1-aminocyclopropane-1-carboxylate (ACC) deaminase, that regulates ethylene levels in plants (Ma et al., 2016a). Ethylene is a phytohormone that plays an important role in plant growth and development and its synthesis depends on ACC synthase. However, under stress conditions, such as metal toxicity, plants end up producing extreme amounts of ethylene which can negatively affect, for instance, plant metabolisms and performance (Manoj et al., 2020). Therefore, ACC deaminase is crucial to normalize ethylene levels and protect plants (Ma et al., 2016a).

3.3. Antibiotics, enzymes, siderophore production and induced systemic resistance

There is a great number of antibiotic compounds produced by PGPR strains that stimulate plant defense mechanisms against a variety of pathogens (Glick, 2012). This trait is effective in preventing the proliferation of other bacteria and fungi (Novo et al., 2018). Plant growth-promoting rhizobacteria are also known to produce hydrogen cyanide, which, despite its reduced individual biocontrol activity, enhances the effect of bacterial antibiotics when acting synergistically (Beneduzi et al., 2012; Glick, 2012). Other important biocontrol agents produced by PGPR are lytic enzymes (such as proteases and cellulases) (Manoj et al., 2020; Novo et al., 2018). These enzymes avoid the proliferation of pathogenic fungi by hydrolyzing components in their cell wall, reducing their populations (Glick, 2012). Besides biocontrol, lytic enzymes are involved in nutrient recycling through organic matter decomposition, leading to increased nutrient availability in the rhizosphere (Karthik et al., 2017). Siderophores produced by PGPR, play a role in Fe sequestration, as aforementioned, and can also contribute to biocontrol activity (Glick, 2012). Bacterial strains use siderophores for preventing pathogens (generally fungi) from acquiring sufficient amounts of Fe, thus hindering their proliferation (Rajkumar et al., 2010).
Plant growth-promoting rhizobacteria are able to induce plant resistance against a large array of pathogens, such as bacteria, fungi, viruses and pests through ISR (Lugtenberg and Kamilova, 2009). Induced systemic resistance is activated by different signaling pathways which include a great variety of molecules (Novo et al., 2018). This mechanism is responsible for a faster and more efficient reaction against pathogens (Beneduzi et al., 2012). Lastly, some biocontrol strains simply outcompete pathogens by efficiently colonizing the plant rhizosphere and consuming resources; this way, the use of most available nutrients makes it difficult for pathogens to grow (Whipps, 2001).

4. Mycorrhizal fungi

Mycorrhiza is described as the mutualistic symbiotic association between soil-borne fungi and the roots of plants (Sieverding et al., 1991). Mycorrhizal fungi are widespread and form associations with the great majority of plants. A characterizing trait of these fungi is that their mycelium overgrows the soil surrounding plant roots (Turnau et al., 2005). The extensive hyphal net around the roots is responsible for significantly increasing the volume of soil that can be explored for nutrients (Thijs et al., 2017). For efficient nutrient uptake, most plants establish a symbiotic relation with mycorrhizal fungi that play a crucial role in supplying important nutrients, augmenting drought tolerance and increasing plant yields and health (Bonfante and Anca, 2009). Promotion of plant growth can be accomplished by enhancing nutrient availability and, via the production of phytohormones, mycorrhizal fungi can also be responsible for protecting plants and enhancing their resistance against biotic and abiotic stress (Waqas et al., 2012). Mycorrhizal fungi have been reported to enhance metal solubilization by producing several organic compounds such as citric and oxalic acid (Coninx et al., 2017). Several types of mycorrhizas have been described, and they can be mainly classified as endomycorrhizas or ectomycorrhizas (Bonfante and Anca, 2009). The main type of endomycorrhizas is formed by arbuscular mycorrhizal fungi (AMF), which penetrate root cells and establish intracellular symbioses (Turnau et al., 2005). In ectomycorrhizas the mycelium forms a somewhat compact fungal mantle on the surface of the roots of trees and shrubs (Turnau et al., 2005).

Arbuscular mycorrhizal fungi can be found in natural and agricultural ecosystems, including heavy metal rich sites (Sheoran et al., 2016). Arbuscular mycorrhizal fungi are incredibly widespread among plant taxa, occurring in 80% of plant species, being by far the most studied type of fungi in hyperaccumulator plants (Göhre and Paszkowski, 2006; Schussler et al., 2001). In this association, the plant is responsible for supplying C to the fungi, while AMF enhance the plant ability to uptake important nutrients, leading to increased plant yields (Miransari, 2011). These fungi comprise an important component that significantly improves the ecosystem efficiency (Miransari, 2011), since they promote soil structure, enhance resistance to pathogens and alleviate stress (such as
metal toxicity) (Miransari, 2011; Turnau et al., 2005). There is no consensus about the mechanisms used by fungi to avoid metal phytotoxicity, meaning that there is the possibility that it might happen indirectly, through the facilitation of nutrient uptake in adverse metal rich environments, stimulating plant development (Kidd et al., 2018). It is also posited that it may happen directly, by regulation of specific metal tolerance mechanisms (Kidd et al., 2018). There is also no unequivocal way of action of AMF regarding the effect in metal uptake and distribution (Kidd et al., 2018). Reports show that several AMF species can increase the uptake of metals by plants and their translocation from root to shoot (Cao et al., 2008). Yet, some species are known to protect the plant by decreasing metal uptake (Rozpądek et al., 2017).

Ectomycorrhizal fungi may occur in metal rich environments and are considered drivers of ecosystem diversity just like AMF. They play an important role in stimulating plant yields in these adverse environments (Kidd et al., 2018). Ectomycorrhizal fungi can improve plants physiological status and modulate heavy metal tolerance (Luo et al., 2014). In some cases, they are responsible for inducing immobilization of certain metals on the surface of the hyphae (component that forms the mycelium) by complexation of the elements with molecular compounds released in the rhizosphere or by using chemical binding groups in the cell wall (Luo et al., 2014).

5. Microbe-assisted phytomining

As previously stated, some plant rhizosphere beneficial microorganisms, such as PGPR and mycorrhizal fungi, inhabit metal rich areas and contribute for the growth, health and yield of hyperaccumulator plants (Benizri and Kidd, 2018). They may play a role in heavy metal tolerance, availability, uptake and distribution within the plant, making them the focus of many studies in recent times (Benizri and Kidd, 2018). Reports show an improvement in hyperaccumulator plants growth and metal accumulation after soil, seed or plant inoculations (Kidd et al., 2018). The potential benefits of microbes associated with hyperaccumulator plants led to the creation of the concept of microbial-assisted phytomining/phytoextraction (MAP) (Thijs et al., 2016). Microbial-assisted phytomining consists in an agronomical strategy that uses microbes beneficial to hyperaccumulators, to improve phytomining yields, resulting in a revenue increase. This approach aims to enhance metal recovery by inoculating hyperaccumulator plants with plant-associated microorganisms, that are able to increase the harvestable amount by enhancing the plants biomass yield and/or the bioavailability, uptake and translocation of the target metal (Benizri and Kidd, 2018). What’s more, microbial-assisted phytomining could replace or complement other agronomical optimization approaches, such as the application of fertilizers or chelating agents, reducing the operational costs.

5.1. Microbe-assisted nickel phytomining
For the effective application of plant beneficial microbiota to enhance Ni phytomining, it is fundamental to have a thorough understanding of the variety, structure and functions of microbes inhabiting the rhizosphere of hyperaccumulators from ultramafic soils (Thijs et al., 2017). Several studies were conducted over the years with a number of PGPR (Table 2) and mycorrhizal fungi, under metal rich substrates and with Ni hyperaccumulator species.

Reports show that bacterial strains collected from ultramafic soils, associated to Ni hyperaccumulators, tolerate much higher concentrations of Ni in comparison to strains from other soils (Schlegel et al., 1991; Turgay et al., 2012). In 2009, Ma et al., studied the effects of several strains of PGPR on plant growth and Ni uptake by the species Brassica juncea and Brassica oxyrrhina (Ma et al., 2009a). The results were positive for IAA production and P solubilization, and almost all strains exhibited siderophore production. Some PGPR also showed ACC deaminase activity. The inoculation of the bacterial strains increased the biomass yield of both plant species, with strains Pseudomonas sp. SR12, Psychrobacter sp. SRS8 and Bacillus sp. SN9 showing the best results. SN9 was also responsible for enhancing Ni concentrations in the root and shoot of both plant species. It was concluded that the inoculation with the bacterial strain SN9 increases Ni accumulation directly and indirectly (by promoting biomass production) in the species B. juncea and B. oxyrrhina. Another study from 2016, demonstrates that the PGPR Bacillus subtilis strain SJ-101, has the ability to promote Ni accumulation, tolerance and growth of the plant B. juncea (Zaidi et al., 2006). It was determined that this strain produced IAA and solubilized P.

Regarding Ni hyperaccumulator species, a study from 2003 reported that Microbacterium arabinogalactonanolyticum, has an important role in enhancing Ni availability and therefore its accumulation by Alyssum murale (Abou-Shanab et al., 2003). Inoculation resulted in an increase of 32.4% in shoot Ni concentration, in comparison with the uninoculated specimens. Another study, with the same plant species, but with the inoculation of the another bacterial strain, Microbacterium oxydans AY509223, also reported a rise in Ni accumulation (Aboushanab et al., 2006). The bacteria were tested on plants in low, medium and high Ni contaminated soils. Results showed that M. oxydans AY509223 enhances foliar Ni in these soils from 82.9, 261.3 and 2829.3 mg kg\(^{-1}\) to 129.7, 430.7 and 3914.3 mg kg\(^{-1}\), respectively, when compared to the control plants. The authors concluded that bacteria are important for hyperaccumulation and the use of inoculants can be developed for improving commercial Ni phytomining.

In 2016, Durand et al., isolated Variovorax strains, from an ultramafic rhizosphere of the mixed cover of Bornmuellera tymphaea - Noccaea tymphaea and Bornmuellera tymphaea – Alyssum murale (Durand et al., 2016). They reported that the total and shoot biomass were significantly superior in the inoculated plant association B. tymphaea – N. tymphaea. The PGPR significantly increased the accumulation of Ni in
roots and shoots, when compared to uninoculated plants. This effect was attributed to the production of IAA and ACC deaminase. Concerning Ni accumulation in the shoots, there was a 39.9 and 79.6% increase in *B. tymphaeae* – *A. murale* and *B. tymphaeae* – *N. tymphaeae*, respectively, when inoculated with bacterial strains. The combination *B. tymphaeae* – *N. tymphaeae* inoculated with *Variovorax* NB24 presented interesting results to be tested in the field.

In 2017, the strains *Arthrobacter* sp. LA44, *Arthrobacter* sp. SBA82 and *Variovorax paradoxus* AB30, were assessed in a field-scale phytomining study with the species *A. murale* (Pardo et al., 2017). The results showed that inoculated plants increased the soil cation-exchange capacity and organic C content, and SBA82 and LA44 did not alter the composition of soil bacterial communities. These strains were responsible for increasing plant coverage and the latter also enhanced significantly the aboveground biomass of *A. murale*. The harvestable amount of Ni per plant was significantly higher with the inoculation of LA44 and SBA82, i.e., the use of these strains has the potential to improve phytomining systems, without compromising the structure of soil bacterial communities.

Table 2 – Summary of some studies with plant growth-promoting rhizobacteria.

<table>
<thead>
<tr>
<th>PGPR strains</th>
<th>Plant species</th>
<th>Mechanism</th>
<th>Main effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Microbacterium arabinogalactanolyticum</em></td>
<td><em>Alyssum murale</em></td>
<td>Siderophore production</td>
<td>↑ Ni availability&lt;br&gt;↑ Ni accumulation</td>
<td>(Abou-Shanab et al., 2003)</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> SJ-101</td>
<td><em>Brassica juncea</em></td>
<td>IAA production&lt;br&gt;P solubilization</td>
<td>↑ Ni accumulation&lt;br&gt;↑ Metal tolerance&lt;br&gt;↑ Biomass</td>
<td>(Zaidi et al., 2006)</td>
</tr>
<tr>
<td><em>Microbacterium oxydans</em> AYS09223</td>
<td><em>Alyssum murale</em></td>
<td>P solubilization&lt;br&gt;Siderophore production</td>
<td>↑ Ni shoot</td>
<td>(Aboushanab et al., 2006)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. Ps29C&lt;br&gt;<em>Bacillus megaterium</em> Bm4C</td>
<td><em>Brassica juncea</em></td>
<td>IAA production&lt;br&gt;P solubilization&lt;br&gt;Siderophore production&lt;br&gt;ACC deaminase activity</td>
<td>↑ metal tolerance&lt;br&gt;↑ Biomass</td>
<td>(Rajkumar and Freitas, 2008)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. SRI2&lt;br&gt;<em>Psychrobacter</em> sp. SRS8&lt;br&gt;<em>Bacillus</em> sp. SN9</td>
<td><em>Brassica juncea</em>&lt;br&gt;<em>Brassica oxyrrhina</em></td>
<td>IAA production&lt;br&gt;P solubilization&lt;br&gt;Siderophore production&lt;br&gt;ACC deaminase activity</td>
<td>↑ Ni root and shoot&lt;br&gt;↑ Biomass</td>
<td>(Ma et al., 2009a)</td>
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<td><em>Psychrobacter</em> sp.&lt;br&gt;SRA1/SRA2&lt;br&gt;<em>Bacillus cereus</em> sp.&lt;br&gt;SRA10/SRP4&lt;br&gt;<em>Bacillus weihenstephanensis</em> SRP12</td>
<td><em>Brassica juncea</em>&lt;br&gt;<em>Brassica oxyrrhina</em></td>
<td>IAA production&lt;br&gt;P solubilization&lt;br&gt;Siderophore production&lt;br&gt;ACC deaminase activity</td>
<td>↑ Ni root and shoot&lt;br&gt;↑ Biomass&lt;br&gt;↑ Availability</td>
<td>(Ma et al., 2009b)</td>
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A pot study was conducted to evaluate the effects of the concurrent use of bacterial inoculation and soil fertilization or soil amendment with cow manure, on Ni phytoextraction by *Odontarrhena bracteata*, *O. Odontarrhena inflata* and *Odontarrhena serpyllifolia* (Ghasemi et al., 2018). For this purpose, serpentine soil from the Melide ultramafic complex (NW Spain), was inoculated with 5 strains of rhizobacteria, previously isolated from *O. serpyllifolia*, and the plants were allowed to grow for three months, before harvesting and analysis. The results showed greater biomass yield, enhanced nutritive conditions, and higher accumulation of Ni in plant tissue. The authors noted that selected bacterial inoculants improved Ni phytoextraction as consequence of stimulated plant growth and/or increased Ni levels in aerial plant parts, although contingent to plant species, type of soil and inoculant. In at least one soil treatment, *Pseudoarthrobacter oxydans* strain SBA82 boosted the shoot dry mass yield of all plant species, whereas *Stenotrophomonas* sp. strain MA98 increased the growth of *O. bracteata* and *O. serpyllifolia*. Plants subjected to rhizobacterial inoculation displayed reduced activity of antioxidative enzymes, and lesser levels of oxidative stress metabolites, denoting an improved physiological response. The authors emphasized the potential of the joint application of rhizobacteria and manure to ameliorate the growth, development, and Ni uptake ability of *Odontarrhena* hyperaccumulators.

Lopez et al. (2019) studied the rhizosphere bacteria of several Ni hyperaccumulator species from the ultramafic soils of Albania. The investigated plant species were *Noccaea ochroleuca*, *Odontarrhena smolikana*, *Odontarrhena rigida* and *Odontarrhena chalcidica*. The study showed that Proteobacteria, Actinobacteria and Acidobacteria were the predominant bacterial phyla in the soil. This work was successful in characterizing the rhizosphere microbial community of hyperaccumulator species and in evaluating their potential influence in soil biogeochemical parameters. The authors suggested the future step to be the determination of traits that allow the bacteria to colonize ultramafic environments, providing significant advantages for sustainable hyperaccumulator production and the enhancement of the phytomining process.
Before the early 2000s hyperaccumulator plants were thought to be non-mycorrhizal, therefore, regarding the application of mycorrhizal fungi for enhancing hyperaccumulation yields, there is still little information in comparison to studies on bacterial inoculants (Benizri and Kidd, 2018). The first Ni hyperaccumulator plant in which the presence of AMF was reported was Berkheya coddii (Turnau and Mesjasz-Przybylowicz, 2003). In this study, several Ni hyperaccumulator species from ultramafic soils in South Africa were analyzed for AMF root colonization. It was found that the plants were all arbuscular mycorrhizal. Considering the species B. coddii, since it exhibits great potential in the context of phytomining, well developed arbuscular mycorrhizas were observed. All plant species inoculated with AMF in greenhouse trials showed a higher biomass production and Ni content in relation to the uninoculated plants.

A study from 2011 consisted in the analyses of the effect of AMF in B. coddii growth and element uptake (Orłowska et al., 2011). The plants were grown in the laboratory on ultramafic soils, and the AMF used in this experiment were from several different origins. Results showed that inoculations affected significantly plant growth and survival, especially with the application of an indigenous strain. The fungi were responsible for increasing concentrations of K, Fe, P and calcium (Ca) in the shoots and Zn, manganese (Mn), Ca and P in roots. The levels of Mn, Co and Ni decreased in plant shoots. However, due to the high biomass production with AMF inoculations, the Ni harvestable amount was much higher (20-fold greater) in mycorrhizal plants when compared to non-mycorrhizal controls. The authors proposed that AMF may help boosting Ni phytomining.

6. Future perspectives

The rhizosphere microbiome, particularly PGPR and mycorrhizal fungi, can play a pivotal role in enhancing plant growth, health and metal uptake. Nonetheless, there is still a wealth of information to be uncovered about rhizosphere microbial diversity, functions and the mechanisms affecting hyperaccumulator plants. Hence, future studies focusing on the influence of mycorrhizal fungi on hyperaccumulators and the scrutiny of the mechanisms responsible for enhanced Ni extraction would be particularly relevant, since the information about these subjects is relatively scarce. The number of bacterial strains examined to date is also quite limited. Hence, further phytomining efforts involving hyperaccumulators and PGPR would certainly provide valuable insights. Field trials are of particular importance for these deliver results under real-world conditions and facilitate a deeper understanding of the processes involved, without tampered variables. Several central aspects such as the compatibility of the selected host species and inoculant, degree of plant species-specificity and the ability of microbes to perform and proliferate in the soil, need to be studied under natural field conditions for longer time periods, since they dictate the efficiency of inoculants (Kidd et al., 2018). Furthermore, inoculation methods like the frequency, volume and timing of inoculation,
and the application in soil, seed or plant, need to be considered because these are aspects that are likely to influence the outcome of phytomining yields. It is also important to assess the effects of inoculants in the natural rhizosphere microbiome.

Lastly, phytomining could also profit from the application of advanced gene editing techniques like clustered regularly interspaced short palindromic repeats (CRISPR) to obtained more efficient or tailor-made PGPR (Basharat et al., 2018). Given that genes responsible for the synthesis of phytohormones, nitrogenase, and siderophores have been already identified, the prospect of designing PGPR with improved direct (phytohormones production, phosphate solubilization, N fixation, and Fe sequestration) and indirect (biocontrol) mechanisms may not be so far-fetched.

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