

Plants as extreme environments? Ni-resistant bacteria and Ni-hyperaccumulators of serpentine flora

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Abstract During recent years there has been an increasing interest in the bacterial communities occurring in unusual, often extreme, environments. On serpentine outcrops around the world, a high diversity of plant species showing the peculiar features of metal hyperaccumulation is present. These metal hyperaccumulators have received much attention for their potential biotechnological exploitation in phytoremediation processes, but also as unusual, extreme habitats for the associated bacterial flora, which could reveal novel details concerning bacterial adaptation. This paper will briefly focus on the research topics that have been addressed to date on bacteria associated with serpentine plants and aims to

provide a state of the art and to present possible future directions for research which could lead to new insights on microbial adaptation and evolution, and potentially applied in technologies for sustainable use and remediation of contaminated land.

Keywords Bacterial communities · Heavy-metals · Hyperaccumulators · Nickel resistance · Serpentine soil

Background

Serpentine soils are distributed all over the world. They originate from an array of ultramafic rock types of hydrous magnesium iron phyllosilicate minerals. Serpentine-rich rock has an olive greenish-gray color, usually blotched with stripes of different shades looking like the skin of a snake (*serpens* in latin), from which the name serpentine is derived. Serpentine rocks originate from metamorphic alterations of peridotite and pyroxene with water and may form near the earth surface, or in the upper part of the earth mantle during subduction events. These rocks, and the soils derived from them, are characterized by high levels of nickel, cobalt and chromium, low levels of N, P, K, Ca, and a high Mg/Ca ratio (Brooks 1987). Such chemical properties render serpentine soils particularly toxic and unsuitable for most plant species (Brady et al. 2005; Chiarucci et al. 1998) and for many microorganisms (Lipman 1926). Ser-

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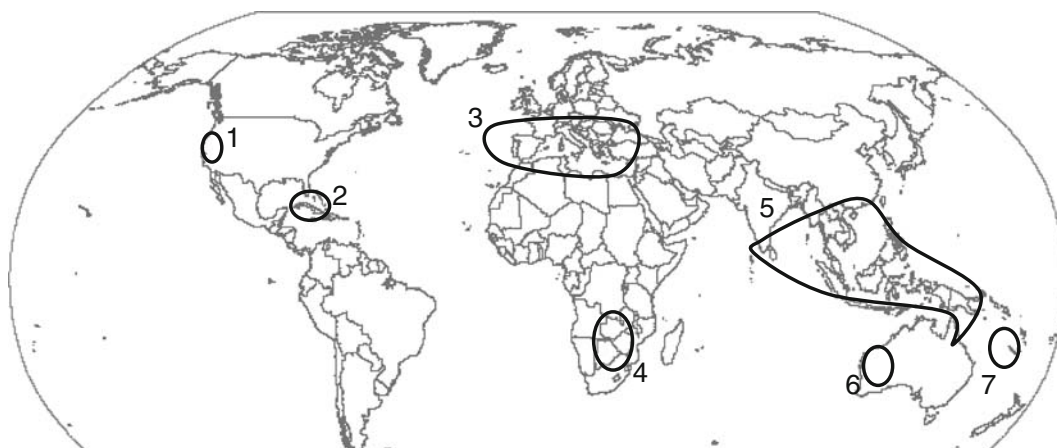
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serpentine outcrops are actually covered by a thin soil layer, with a tenuous vegetation and, consequently, a low organic matter content.

Plant biologists have studied in depth the ecology, physiology, phylogeny and taxonomy of plants occurring on serpentine soils (serpentinophytes, for a review see Brady et al. 2005), and described a high number of endemic taxa, often with unusual features, such as metal hypertolerance or metal hyperaccumulation, a puzzling phenotype consisting of extremely high foliar metal contents, probably as a defense against herbivory (Boyd 2007) (Fig. 1). In an evolutionary perspective, serpentine soils might be considered, similar to other metal contaminated soils, as “ecological islands” (Lefèbvre and Vernet 1990), inhabited by particular, often endemic, taxa. The ecological island model has boosted much research on evolution and adaptation and provoked discussion on the microevolutionary dynamics of metal tolerance and metal hyperaccumulation in plants, from the population to the single-gene level (for examples see

Berglund et al. 2004; Kazakou et al. 2008; Mengoni et al. 2003a; Mengoni et al. 2003b; Rajakaruna et al. 2003; Vekemans and Lefèbvre 1997). Despite the long history of interest in serpentine plants, the attention of microbiologists towards bacteria from serpentine habitats is more recent, with the relevant exception of Lipman (1926), and intimately linked to the peculiar botanical features of serpentine outcrops. Although many plant-associated bacteria have been demonstrated to have beneficial effects on their host, their importance is still underestimated. A better understanding of their growth-promoting effects could be exploited for sustainable growth of crops, biomass for biofuel production, and feedstock for industrial processes, even on metal contaminated land (Weyens et al. 2009b; Rajkumar et al. 2009b). Furthermore, plant-associated bacteria can be exploited to improve the efficiency of phytoremediation processes. Very recently, a review paper has been published on the biotechnological applications of serpentine soil bacteria (Rajkumar et al. 2009a). The present paper aims



Number	Area	Some representative Ni-hyperaccumulators	References
1	Northern California/Oregon	<i>Streptanthus polygaloides</i> , <i>Thlaspi montanum</i>	Brooks 1987
2	Cuba	<i>Phyllanthus discolor</i> , <i>Phyllomelia coronata</i>	Reeves et al. 1999
3	Southern Europe/Asia Minor	<i>Alyssum</i> (several species), <i>Bornmuellera</i> (syn. <i>Ptilotrichum</i>) <i>baldaccii</i> , <i>Thlaspi goesingense</i>	Brooks 1987
4	Zimbabwe/Zambia/Zaire/South Africa	<i>Berhkeya coddii</i> , <i>Senecio coronatus</i>	Anderson et al. 1997; Boyd et al. 2002
5	South-East Asia	<i>Myristica laurifolia</i> , <i>Rinorea bengalensis</i> , <i>Walsura monophylla</i>	Brooks 1987, Baker et al. 1992
6	Western Australia	<i>Stackhousia tryonii</i>	Batianoff et al. 1990
7	New Caledonia	<i>Sebertia acuminata</i> , <i>Xylosma</i> (several species)	Brooks 1987

Fig. 1 Worldwide distribution of serpentine outcrops where Ni-hyperaccumulators have been found (modified from Brooks 1987). Black lines approximately encircle areas where serpentine

outcrops are present. The table reports the name of the geographical areas with some of the Ni-hyperaccumulators found

to complement and integrate the above-mentioned review, focusing on the adaptation and ecology of serpentine plant-associated bacteria. Possible future research perspectives that could deliver new insights concerning microbial adaptation and evolution will be identified and discussed.

Bacterial communities in serpentine soil

Bacteria associated with plants are a direct subset of the bacteria present in soil (Rosenblueth and Martinez-Romero 2006; Ryan et al. 2008). But are serpentine soil bacteria endemics, as serpentine plants are? In other words, is it possible to identify a specialized and endemic serpentine bacterial flora, like for plants?

The first evidence that serpentine soil bacterial communities show marked differences with non-serpentine ones came from the pioneering study by Lipman (1926) who, in an attempt to identify the reasons for the low fertility of serpentine soils, wrote: “*there is little diversity, as well as a general paucity, in the bacterial flora of the serpentine soils.*”

It still is not clear if serpentine soils harbour a characteristic bacterial taxonomic diversity, that is, if certain bacterial taxonomic groups are deprived or favored by the serpentine soil conditions. To our knowledge only one paper (Oline 2006) reported a comparative analysis of 16S rRNA gene libraries of serpentine and non-serpentine soils. However, this study did not provide a definitive answer. Moreover, no extensive studies were done on the functionality of soil microbial communities and on the effectiveness of biogeochemical cycles in which bacteria are involved. Up to now, it has only been shown that both the metal-resistant and the total bacterial flora of bulk serpentine soil are mainly composed of *Actinobacteria* and *Proteobacteria* (Lodewyckx et al. 2002b; Mengoni et al. 2001; Oline 2006), the first ones being the dominant group in cultivation-independent analyses. Considering that serpentine soil is toxic to microorganisms, partly because of the metals present, soils polluted by metals due to industrial processes might be taken to resemble serpentine soils. In this perspective, similarly to what was found in serpentine soils in California (Oline 2006), an analysis of clone libraries from 16S rDNA and 16S rRNA, amplified from an anthropogenic

polluted soil in Switzerland (Gremion et al. 2003), showed that sequences from *Actinobacteria*, *Acidobacteria*, *alpha-* and *beta-Proteobacteria* were dominant, while only few members of *Firmicutes* were found. However, as shown by Oline (2006), the same taxonomic divisions are also dominant in non-serpentine soils surrounding the serpentine outcrops; therefore, definitive conclusions cannot be drawn from these data. Consequently, more extensive studies on many different serpentine outcrops and on non-metalliferous comparable soils (e.g. with low organic matter and high Mg/Ca ratio), analyzing high numbers of 16S rRNA libraries with next generation sequencing technologies (Andersson et al. 2008; Droege and Hill 2008; Keijser et al. 2008), should be performed to clarify if serpentine soils harbor significantly different taxonomic composition respective to non-metalliferous soils. Finally, given that a plant species has a completely different ecological and evolutionary connotation than a bacterial species, due to the high incidence of horizontal gene transfer (Gevers et al. 2005; Konstantinidis et al. 2006; Pace 2009; Staley 2006), these analyses may not necessarily show any taxonomic exclusiveness of serpentine soil: adaptation to serpentine in bacteria could be much more a matter of genes than of taxa. In the last paragraph we will discuss and review some data which will allow to shift our attention more to gene functions than to bacterial taxa.

Plants from serpentine soil provide ecological niches for metal resistant bacteria

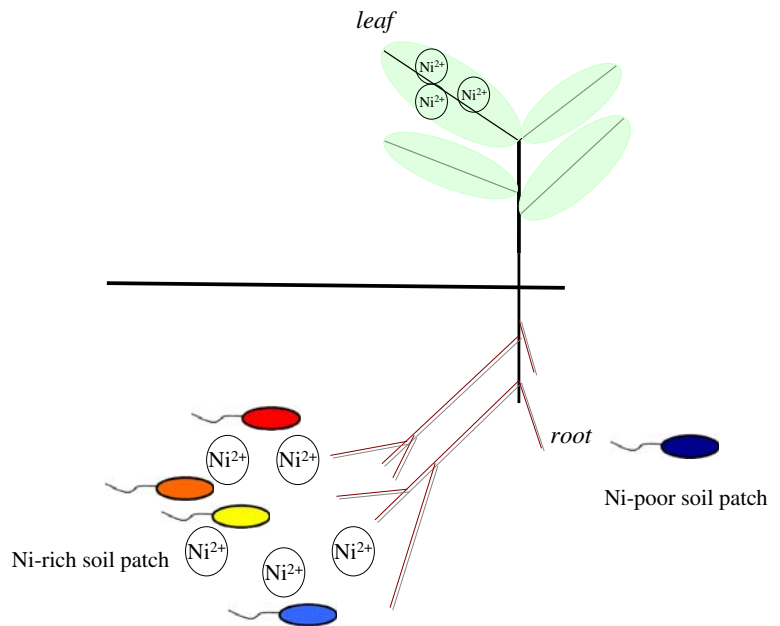
After the above-cited paper by Lipman (1926), the true beginning of serpentine microbiology can be traced back to 1991, when a collaborative paper by Hans Schlegel and co-workers reported a first analysis of bacteria present in the serpentine soil of New Caledonia (Schlegel et al. 1991). They analyzed the effect of an astonishing Ni-hyperaccumulator tree, *Sebertia acuminata* (Sagner et al. 1998), which contains up to 25000 mg kg⁻¹ of nickel in its leaves (Perrier et al. 2004), on the bacteria present in the soil underneath its canopy. They found that the presence of the plant positively correlated with the presence of Ni-resistant soil bacteria. A higher proportion of Ni-resistant bacteria was in fact recovered in proximity of the plant, suggesting the presence of a hypothetical

“nickel cycle”, driving the evolution of the bacterial community towards a higher percentage of nickel-resistant strains. The “nickel cycle” leads to an increased nickel concentration in the upper soil layers in the proximity of the plant due to the “pumping” of nickel from deep soil performed by the roots, followed by the translocation of nickel to leaves and then, after the abscission of the leaves, the release of accumulated nickel from the litter. As a consequence of this cycle, top soil layers near the plant contain higher nickel concentrations than top soil layers far away from the plant, and consequently exert a stronger selective pressure for Ni-resistance towards soil bacteria. This phenomenon stirred the attention of other investigators, who looked for the occurrence of the same phenomenon in other serpentine plant species, such as *Alyssum bertolonii* and *Thlaspi caerulescens*. Recently, an increased fraction of Ni-resistant bacteria was also observed in the rhizosphere of the Ni-hyperaccumulators *A. bertolonii* (Mengoni et al. 2001), *Thlaspi goesingense* (Idris et al. 2004) and *A. serpyllifolium* susp. *lusitanicum* (Becerra-Castro et al. 2009). Likewise, an enhanced fraction of Zn-resistant bacteria was found in the rhizosphere of the Zn-hyperaccumulator *T. caerulescens* (Aboudrar et al. 2007; Lodewyckx et al. 2002a). However, due to the small size and shallow rooting of these plants, it is probably not correct to invoke a real “metal cycle”, that is, an increase of the top soil metal concentration due to the foliar hyperaccumulation of deep-soil metals and subsequent leaf fall. Also, the ability of hyperaccumulators to mobilize more nickel or zinc from soils or rocks does not seem to be higher than in normal plants (Bernal et al. 1994; McNear et al. 2007; Puschenreiter et al. 2005; Shallari et al. 2001). A possible hypothesis for the presence of highly tolerant bacteria near the roots of metal hyperaccumulators could be related to the specific tropism, shown in controlled experiments, of roots of hyperaccumulating plants toward soil patches rich in metals, a phenomenon known as “root metal foraging” (Haines 2002; Liu et al. 2009; Schwartz et al. 1999; Whiting et al. 2000). Consequently, the presence of highly tolerant bacteria near plants roots may not be due to plant activity but simply to the chemical parameters of the soil patch that already selected a highly tolerant bacterial flora (Fig. 2). Moreover, it has been shown that rhizosphere bacteria may contribute to mineral solubilisation (Abou-

Shanab et al. 2003a), thus eventually increasing metal bioavailability in soil patches (Doty 2008; Lebeau et al. 2008; Weyens et al. 2009b). However, to date no reports clearly suggest that hyperaccumulating plants may select metal mobilizing bacterial strains for tight association with roots in a type of metal-for-carbon symbiosis. However, despite several reports on the rhizosphere of metal-hyperaccumulators, there are no data on the bacteria associated with the roots of non-hyperaccumulators growing in the same environments. Theoretically, if rhizosphere bacteria are able to solubilise metal specifically due to their metabolic activities, one could find an increased proportion of metal tolerant bacteria, in comparison with the bulk soil, also in the rhizosphere bacterial community of non-hyperaccumulating serpentinophytes. We think that this point has to be seriously addressed, in order to establish the evolutionary scenario and the physiological role of the bacterial community associated with metal-hyperaccumulators. Good models for such tests could be species of the genus *Alyssum* which have populations showing both hyperaccumulation and non-accumulation, such as *A. peltarioides* and *A. sibiricum* (Mengoni et al. 2003c). However, investigation of the bacterial flora associated with other serpentinophytes not belonging to the *Brassicaceae* family should be performed to evaluate the potential role of taxonomic biases owing to the over-representation of this family among hyperaccumulators.

Bacterial strains with high metal-tolerance have been isolated from hyperaccumulator rhizospheres (Abou-Shanab et al. 2007a; Abou-Shanab et al. 2003b; Idris et al. 2004; Pal et al. 2007), of which some have been shown to contribute to plant growth and, occasionally, to plant metal accumulation (Abou-Shanab et al. 2008; Abou-Shanab et al. 2006; Li et al. 2007; Rajkumar and Freitas 2008a, b; Sheng et al. 2008; Weyens et al. 2009b). A recent book chapter (Sessitsch and Puschenreiter 2008) highlighted the importance of research on bacteria associated with plant hyperaccumulators to develop new strategies for improving current phytoremediation techniques. To date, some of the technological improvements of plant metal accumulation and tolerance have been performed with genetically modified strains, transferring Ni-resistance determinants from *Cupriavidus metallidurans* or phytochelatin synthase from *Arabidopsis thaliana* into endophytic strains such as *Burkholderia*

Fig. 2 Consequences of “metal root foraging” on the composition of the rhizosphere bacterial flora. Following the root foraging hypothesis (Schwartz et al. 1999), plant roots show a tropism toward soil patches rich in metals. These patches are already inhabited by a larger fraction of Ni-resistant bacteria (shown in different colours to suggest possibly different bacteria strains) than Ni-poor patches, which will then constitute the main part of the rhizosphere bacterial communities. Bacteria are conventionally represented with a flagellum without any speculation on their biological status



cepacia, *Herbaspirillum seropedicae* (Lodewyckx et al. 2001) and *Escherichia coli* (Sauge-Merle et al. 2003). However, the use of recombinant strains may be of limited applicability, owing to biosafety aspects, unless recombinant strains can be obtained using natural gene transfer (Weyens et al. 2009b). In this respect also the exploitation of the biodiversity of natural metal resistant strains associated with plants from serpentine soils, may be very adequate for developing species-specific inoculants to increase the yield of phytoremediation in metal contaminated areas, such as mines, or areas impacted by smelters or other industries, and for phytomining of serpentine outcrops (Chaney et al. 2007; Chaney et al. 2005). As recently reviewed by Rajkumar et al. (2009a), of the few strains isolated in 2003 from *Alyssum murale* (Abou-Shanab et al. 2003a), now a panel of 14 bacterial strains from the rhizosphere of plants grown in serpentine soil have been characterized, displaying plant growth and metal accumulation promoting activities.

Plant endosphere: a new emerging niche for metal resistant bacteria

More recently, the increasing interest of the research community for endophytic bacteria, that is bacteria

intimately associated with plant tissues (Hardoim et al. 2008; Weyens et al. 2009a, b), has opened new directions, still related to plants from serpentine soils. The potential applications of endophytic bacteria are many-fold (Lodewyckx et al. 2002b; Moore et al. 2006; Newman and Reynolds 2005; Ryan et al. 2008; Selosse et al. 2004; Weyens et al. 2009a, b) and the exploitation of bacterial strains and bacterial communities in metal tolerant and metal hyperaccumulating plants could have a direct impact on plant performance (Lodewyckx et al. 2002b). One of the most basic questions concerns the relationships existing between endosphere (endophytic) and rhizosphere bacteria in hyperaccumulators. Endophytic bacteria are thought to enter plant tissue mainly from roots or at sites of wounding (Hurek and Reinhold-Hurek 2003; Reddy et al. 1997; Rosenblueth and Martinez-Romero 2006), but some endophytes seem to be transferred from one plant generation to the next through the seeds (Mastretta et al. 2009). Consequently, bacteria of the endosphere are, at least partly, a subset of rhizosphere and of phyllosphere community. To date it is still not clear if there could be endospheric strains which are propagated exclusively through seeds and are not found in soil. In the case of hyperaccumulators, endophytes may colonize plant-internal environments that are less toxic than soil (that is with lower

available metal contents), or environments, such as xylem vessels, where toxic metals might be available at higher concentrations than in soil (Smart et al. 2007). From this perspective, the Ni-hyperaccumulator serpentine endemic plant *Thlaspi goesingense* has been investigated for its endophytic bacterial community composition (Idris et al. 2004). Clone libraries, Terminal-Restriction Fragment Length Polymorphism (T-RFLP) fingerprintings and plate isolation were used to characterize the rhizosphere and the endosphere (shoot-associated) bacterial flora. Large differences in taxonomic composition were found between rhizospheric and endophytic communities. In particular, the endophytic community was rich in members of the *Proteobacteria* division and a high number of sequences related to the genus *Sphingomonas* were found, similar to other studies on *Thlaspi* and *Alyssum* rhizospheres (Abou-Shanab et al. 2003a; Lodewyckx et al. 2002a). It could be interesting to look for the presence of *Sphingomonas* in the endosphere and rhizosphere of other non-accumulator serpentinophytes to address the presence of specific interactions between hyperaccumulators and *Sphingomonas* strains. Moreover, members of the genus *Methylobacterium* were recovered and one species, named *Methylobacterium goesingense* was found to be associated with the plant (Idris et al. 2006). Recently, in an effort to characterize the variability of leaf-associated community between individual plants of *A. bertolonii* by T-RFLP fingerprinting, we found that, while Terminal-Restriction Fragments (T-RFs) corresponding to *Sphingomonas* were common to both soils and plants, T-RFs identified as *Methylobacteria* were present only in foliar DNA extracts (Mengoni et al. 2009). Moreover, in *Thlaspi* (Idris et al. 2004), more isolates tolerant to higher Ni concentration were found in the plant endosphere than in the rhizosphere, thus reinforcing the hypothesis that Ni-hyperaccumulating plants provide highly selective and peculiar niches for bacteria. In agreement with this finding, endospheres from roots, shoots and leaves of *A. bertolonii* were shown to contain bacterial isolates possessing different levels of Ni-resistance (Barzanti et al. 2007) although the biological reasons for such differences is elusive yet. Notably, isolates from leaves displayed the lowest levels of Ni-resistance compared with those from the other organs in

agreement with a low Ni bioavailability due to vacuolar storage of Ni in epidermal cells and trichomes (Ingle et al. 2008; Kupper et al. 2001; Smart et al. 2007).

Genes for metal resistance: single operons or genome adaptation?

What about the most striking feature of serpentine ecology, that is the resistance to metals, particularly to nickel? As plants are tolerant to Ni and as some of them have coped with Ni in such a smart way to evolve the ability to accumulate it (hyperaccumulators), in a similar way bacteria should have coped with free Ni in the soil and, in particular, with that in plants. Genetic determinants for Ni resistance have been first identified and characterized at the molecular level from *Alcaligenes xylosoxidans* 31A (known also as *Ralstonia eutropha*, then as *Ralstonia metallidurans* and now as *Cupriavidus metallidurans* 31A) and *Alcaligenes eutrophus* CH34 (now known as *Cupriavidus metallidurans* CH34) which were isolated from industrially contaminated sites (Mergeay et al. 1978; Schmidt and Schlegel 1989). *A. xylosoxidans* 31A harbors two genetic determinants (*ncc*, *nre*) on plasmid pTOM9, while *C. metallidurans* CH34 contains one system only (*cnr*) (Liesegang et al. 1993; Mergeay et al. 2003; Nies 2000), but a large number of transporters and a regulatory apparatus are present also in its genome constituting an integrated (both plasmid-borne and chromosomal) system for resistance to metals (Mergeay et al. 2003; von Rozycki and Nies 2008). In all these systems Ni resistance is due to an inducible efflux system, mediated by ATP binding cassette (ABC) transporters, that lower the intracellular Ni concentration by pumping out Ni cations from the cytoplasm (Mergeay et al. 2003). Mergeay et al. (2009) presented a comprehensive review of metal resistance genes in the genus *Cupriavidus*. The presence of these systems was explored in a collection of strains isolated from New Caledonian serpentine soils and several of them (7 out of 22 strains) showed slight or no hybridization signals with probes from Ni resistance determinants of *A. xylosoxidans*, *C. metallidurans*, or other strains from polluted environments such as *A. denitrificans* 4a-2 and *K. oxytoca* 15788 (Stoppel and Schlegel 1995). Among them, *Hafnia alvei* 5-5, isolated from the soil-litter mixture underneath the canopy of the

nickel-hyperaccumulating tree *Sebertia acuminata*, has recently been reported to harbor a system, named *ncr* (Park et al. 2004), which is similar to the *nre* from *C. metallidurans* 31A and *Legionella pneumophila* (Hahm et al. 2002) and to *nir* from *Klebsiella oxytoca* (Stoppel et al. 1995); *ncr*-like elements have also been identified in *Leptospirillum ferriphilum* isolated from a mine in Jiangxi, China (Tian et al. 2007). Recently, a new system, named *nrp*, showing no homologies with known nickel-resistance proteins, has been identified in an *Enterobacter* strain isolated from soil in Korea (Lee et al. 2006). *Nre*-like and *cnr*-like systems have also been found in nickel-resistant *Bradyrhizobium* strains nodulating the legume *Serianthes calycina* in New Caledonian serpentine outcrops (Chaintreuil et al. 2007). In these strains, *cnrA* was demonstrated to confer a selective advantage to both, plants and bacteria, by increasing the number of root nodules over the *cnrA*⁻ mutant strain in soil spiked with 15 mM NiCl₂. In other strains from serpentine soil different systems seem to confer Ni resistance: for instance, in a recently described strain of *Serratia marcescens*, a homolog of the *ncrABC* determinant, plus other systems related to magnesium homeostasis (*mgfC*), were found to be the main mediator of Ni resistance (Marrero et al. 2007). Moreover, additional genes were found in this strain to be involved in determining resistance levels such as the *gidA* gene encoding the glucose-inhibited division protein A, a ribosomal protein and a subunit of elongation factor Tu in translation. It is noteworthy that several molecular mechanisms have evolved, which help these serpentine strains to cope with Ni and probably with the harsh conditions and unusual chemical composition encountered in serpentine soil (high content of transition metals, low Ca and high Mg concentrations, low organic matter, low water content, etc.). Consequently, the exploitation of strains naturally present in association with serpentine plants, more than the use of engineered strains, could be really effective for finding strains well adapted to survive in toxic soils and assisting plants to cope with metals for improving phytoremediation.

In a recent metagenomic analysis of the rhizosphere of plants growing in the banks of Rio Tinto (Mirete et al. 2007), new genetic systems conferring nickel resistance have been identified by screening an *E. coli* library: these include ABC transporters and enzymes, as serine acetyltransferase (SAT), which are

known to play similar roles in hyperaccumulating plants (Freeman et al. 2004). From these data an emerging hypothesis is that for bacteria several independent mechanisms may operate to confer metal resistance, emphasizing the role of the species' (and community's) gene pool and horizontal gene transfer other than that of single genes. Some of the genetic determinants of Ni resistance (Table 1) seem to be involved in a range of normal metabolic pathways (e.g. increased glutathione synthesis by enhanced expression of SAT), but others in more specific systems (e.g. transenvelope efflux proteins) which may independently give rise to the metal-tolerant phenotype. However, more and more strains from serpentine habitats (soil, plant rhizosphere and endosphere) must be analyzed to solve the puzzle: are there well adapted, specialized, bacterial strains, or is the resistance to serpentine environments easily achieved by few mutations in key ABC transporters or by acquisition through horizontal gene transfer of cassettes containing single operons? The main unanswered question is concerned with the evolutionary dynamics of Ni-resistance systems in serpentine habitats: how many different genes are involved and to what extent do they result from recombination with other elements, or horizontal gene transfer?

Perspectives

What is the future of serpentine microbiology? The main questions are related to the evolutionary dynamics and distribution of metal resistance mechanisms over the bacterial community and the role of interactions with serpentinophytes. In other words, in the process of evolution taking place in serpentine soil a) How many different genetic systems have evolved to cope with the same selective pressure and how did these systems evolve? b) Do metal-hyperaccumulators have selected characteristic rhizospheric and endophytic microflora, as compared with non-accumulators? The answers to these questions could provide important clues for evaluating genomic and population contribution to the establishment of complex soil bacterial communities in response to abiotic and biotic parameters, and for the possible application of bacteria to improve phytoremediation practices.

We need more model bacterial species, than the sole *C. metallidurans* CH34, for exploiting the

Table 1 List of genetic determinants conferring Ni-resistance^a

Name	Organism	Location	Habitat of isolation	Reference
Cnr	<i>Cupriavidus metallidurans</i> CH34	Plasmid pMOL28	Sludge (zinc decantation tank, Belgium)	Liesegang et al. 1993
Ncc	<i>Cupriavidus metallidurans</i> 31A	Plasmid pTOM9	Metal contaminated wastes (Germany)	Schmidt and Schlegel 1989; Schmidt and Schlegel 1994
Nre	<i>Cupriavidus metallidurans</i> 31A	Plasmid pTOM9	Metal contaminated wastes (Germany)	Grass et al. 2001
Nir	<i>Klebsiella oxytoca</i> CCUG 15788	Chromosome	Mineral oil emulsion tank (Göteborg, Sweden)	Park et al. 2008; Stoppel et al. 1995
Ncr	<i>Hafnia alvei</i> 5-5	plasmid pEJH 501	Soil-litter mixture (New Caledonian serpentine)	Park et al. 2004
Nrp	<i>Enterobacter</i> sp. Ni15	Plasmid pNi15	Soil (Wonsando, Korea)	Lee et al. 2006
Ncr-like	<i>Leptospirillum ferriphilum</i> UBK03	Not known	Mine soil (Jiangxi, China)	Tian et al. 2007
	<i>Serratia marcescens</i> C-1	Not known	Serpentine soil (Cuba)	Marrero et al. 2007
Cnr-like	Several <i>Bradyrhizobium</i> strains	Not known	Rhizosphere soil (New Caledonian serpentine)	Chaintreuil et al. 2007
Nre-like	Several <i>Bradyrhizobium</i> strains	Not known	Rhizosphere soil (New Caledonian serpentine)	Chaintreuil et al. 2007
	<i>Synechocystis</i> sp. PCC 6803	Chromosome	Water (freshwater lake)	Kaneko et al. 1996
	<i>Arthrobacter aurescens</i> TC1	Plasmid TC2	Soil contaminated with the herbicide atrazine	Mongodin et al. 2006
SAT	Metagenomic library	Not known	Rhizosphere soil (Rio Tinto banks)	Mirete et al. 2007

^a The name of the genetic determinant, the organisms or the source of isolation, the genomic location of the determinant and the reference of the original isolation are reported

genomics of metal tolerance in bacteria. Very promising strains have been isolated from hyperaccumulators and from bulk serpentine soil, which could be good models for serpentine genomics, as for instance *Methylobacterium goeisingense* (Idris et al. 2006), some strains from the rhizosphere of *Alyssum murale* (Abou-Shanab et al. 2007a; Abou-Shanab et al. 2003b), but also *Serratia marcescens* C-1 (Marrero et al. 2007) or *Streptomyces yatensis* (Saintpierre et al. 2003). Genome sequencing of such strains could provide new important hints for the evolutionary aspects of metal resistance and for unravelling the genetic basis of their positive interactions with hyperaccumulating plants. Moreover, serpentine soils could be good models for metagenomics due to the expected low number of species, in comparison with agricultural or forest soils, and may provide many gene functions for industrial biotechnology applications. It has recently been shown (Quince et al. 2008) that an estimate for a soil metagenome would require a tremendous effort, compared with ocean metagenomics. For instance, the international consortium

“Terragenome” (<http://www.terragenome.org>), which is aimed at deciphering at large scale the metagenome of a reference soil, is in fact an ongoing effort which groups scientist from 23 countries and will constitute one of the largest genomic investigations of the next years. Moreover, similar to the rice endophyte metagenome project (<http://www.jgi.doe.gov/genome-projects/>), the sequencing of the endophytic metagenome of metal hyperaccumulators and non-accumulators could greatly increase our understanding of plant-bacterial relationships and of evolution at the gene function level, and could be helpful in finding inoculants and target genes for improving plant metal tolerance and metal hyperaccumulation for phytoremediation and phytomining applications (Rajkumar et al. 2009a). In fact, metal availability, metal uptake and phytotoxicity for the plant are the main limiting factors for the application of phytoextraction (Weyens et al. 2009a, b). Exploitation of plant-associated bacteria could be a promising strategy to improve the efficiency of phytoextraction both *via* enrichment of the bacterial community present *in*

planta (Abou-Shanab et al. 2007b; Li et al. 2007; Sheng et al. 2008; Mastretta et al. 2009), or through metabolic engineering and re-inoculation of suitable strains to improve metal availability (e.g. due to the synthesis of natural chelators and acidifying components), and reduce phytotoxicity (e.g. due to bacterial metal sequestration), respectively (Lodewyckx et al. 2001; Sheng et al. 2008; Valls and de Lorenzo 2002).

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