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Phytoremediation: plant–endophyte partnerships take the challenge

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A promising field to exploit plant–endophyte partnerships is the remediation of contaminated soils and (ground) water. Many plant growth promoting endophytes can assist their host plant to overcome contaminant-induced stress responses, thus providing improved plant growth. During phytoremediation of organic contaminants, plants can further benefit from endophytes possessing appropriate degradation pathways and metabolic capabilities, leading to more efficient contaminant degradation and reduction of both phytotoxicity and evapotranspiration of volatile contaminants. For phytoremediation of toxic metals, endophytes possessing a metal-resistance/sequestration system can lower metal phytotoxicity and affect metal translocation to the above-ground plant parts. Furthermore, endophytes that can degrade organic contaminants and deal with or, even better, improve extraction of the metals offer promising ways to improve phytoremediation of mixed pollution.

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Introduction

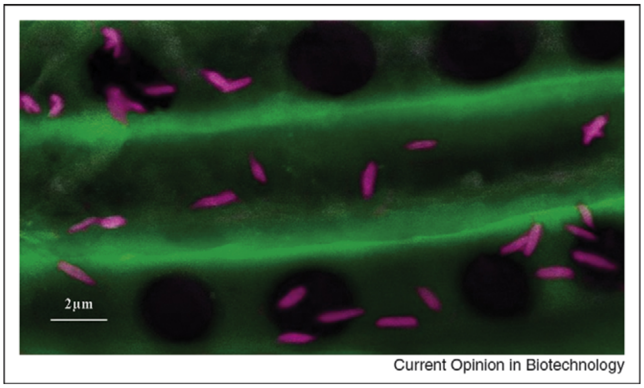
Plants and their associated microorganisms are characterized by varied and complex interactions and have been the subject of extensive research and diverse applications. Endophytic bacteria can be defined as bacteria colonizing the internal tissues of plants without causing symptoms of infection or negative effects on their host [1•]. With the exception of seed endophytes, the primary site where endophytes gain entry into plants is via the roots. Several microscopic studies confirm this route of colonization [2,3•]. Once inside the plant, endophytes either reside in specific plant tissues like the root cortex or the xylem (Figure 1), or colonize the plant systematically by transport through the vascular system or the apoplast [4,5].

Endophytic bacteria have been isolated from a variety of healthy plant species ranging from herbaceous crop plants [6,7•,8,9] and different grass species [10,11] to woody tree species [12–14,15•]. In general, *Pseudomonaceae*, *Burkholderiaceae* and *Enterobacteriaceae* are among the most common genera of cultivable endophytic species found [16].

In comparison with rhizosphere and phyllosphere bacteria, endophytic bacteria are likely to interact more closely with their host. In these very close plant–endophyte interactions, plants provide nutrients and residency for bacteria, which in exchange can directly or indirectly improve plant growth and health (for review see [16]). Direct plant growth promoting mechanisms may involve production of plant growth regulators such as auxins, cytokinins and gibberellins, suppression of stress ethylene production by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, nitrogen fixation and the mobilization of unavailable nutrients such as phosphorus and other mineral nutrients. Endophytic bacteria can indirectly benefit plant growth by preventing the growth or activity of plant pathogens through competition for space and nutrients, production of hydrolytic enzymes, antibiosis, induction of plant defence mechanisms and through inhibition of pathogen-produced enzymes or toxins.

In addition to their beneficial effects on plant growth, endophytes have considerable biotechnological potential to improve the applicability and efficiency of phytoremediation. Phytoremediation (the use of plants and their associated microorganisms to remediate a site) is an *in situ*, solar powered remediation technology that requires minimal site disturbance and maintenance resulting in a low cost and a high public acceptance. Since conventional remediation options currently available are frequently expensive and environmentally invasive, phytoremediation turns out to be a valuable alternative, especially for the treatment of large contaminated areas with diffuse pollution. Large-scale applications of phytoremediation still face a number of obstacles, including the levels of contaminants (being toxic for the organisms involved in remediation), the bioavailable fraction of the contaminants (being too

Figure 1



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Pseudomonas putida colonizing the root (5 cm from the apex) xylem of poplar.

low) and, in some cases, evapotranspiration of volatile organic pollutants from soil or groundwater to the atmosphere. This review describes the potential for exploiting plant–endophyte partnerships to improve phytoremediation of organic contaminants and toxic metals.

Plant–endophyte partnerships in phytoremediation

Plant uptake of organic contaminants

Plant uptake is the first crucial step in whole plant-metabolism of organics. In case of constant plant and environmental features, the lipophilicity of the compound – expressed as its octanol–water partition coefficient (K_{ow}) – was shown to be the determining factor for root entry and translocation. Organic contaminants with a log $K_{ow} < 1$ are considered to be very water-soluble, and plant roots do not generally accumulate them at a rate surpassing passive influx into the transpiration stream [17]. Contaminants with a log $K_{ow} > 3.5$ show high sorption to the roots but slow or no translocation to the stems and leaves [18]. However, plants readily take up organic contaminants with a log K_{ow} between 0.5 and 3.5, as well as weak electrolytes (weak acids and bases or amphoteres as herbicides) (Table 1).

Table 1

log K_{ow} (octanol–water partition coefficient) values of some frequently found organic contaminants.

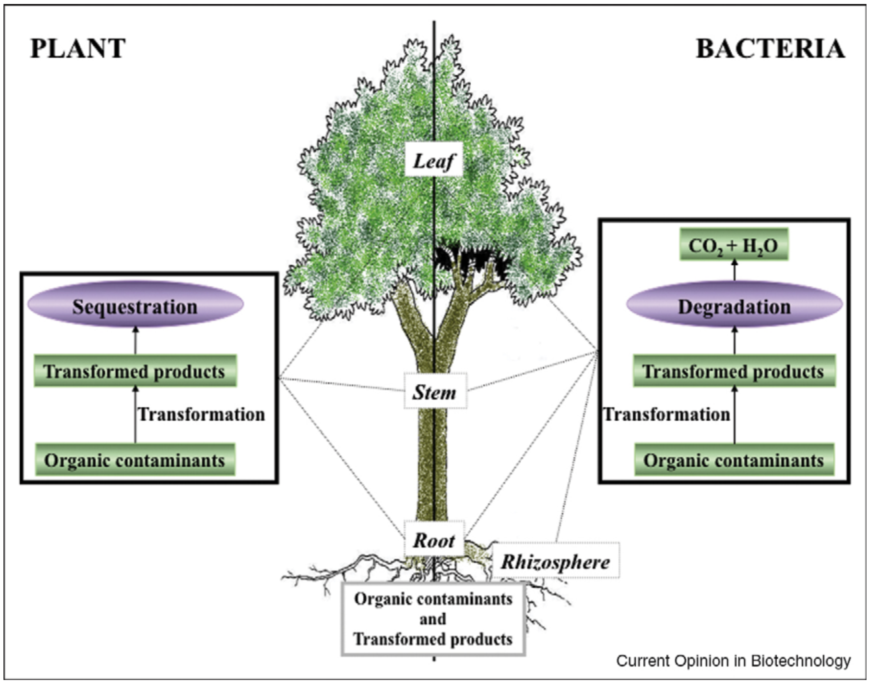
2-Butanone	0.3	3-Chlorobenzoic acid	2.7
4-Acetylpyridine	0.5	Toluene	2.7
Aniline	0.9	1-Naphthol	2.7
Acetanilide	1.0	2,3-Dichloro aniline	2.8
Benzyl alcohol	1.1	Chlorobenzene	2.8
4-Methoxyphenol	1.3	Allyl phenyl ether	2.9
Phenoxyacetic acid	1.4	Bromobenzene	3.0
Phenol	1.5	Ethyl benzene	3.2
2,4-Dinitrophenol	1.5	Benzophenone	3.2
Benzonitrile	1.6	4-Phenyl phenol	3.2
Phenylacetonitrile	1.6	Thymol	3.3
4-Methylbenzyl alcohol	1.6	1,4-Dichlorobenzene	3.4
Acetophenone	1.7	Diphenylamine	3.4
2-Nitrophenol	1.8	Naphthalene	3.6
3-Nitrobenzoic acid	1.8	Phenyl benzoate	3.6
4-Chloraniline	1.8	Isopropylbenzene	3.7
Nitrobenzene	1.9	2,4,6-Trichlorophenol	3.7
Cinnamic alcohol	1.9	Biphenyl	4.0
Benzoic acid	1.9	Benzyl benzoate	4.0
p-Cresol	1.9	2,4-Dinitro-6-sec-butyl phenol	4.1
cis-Cinnamic acid	2.1	1,2,4-Trichlorobenzene	4.2
trans-Cinnamic acid	2.1	Dodecanoic acid	4.2
Anisole	2.1	Diphenyl ether	4.2
Methyl benzoate	2.1	Phenanthrene	4.5
Benzene	2.1	n-Butylbenzene	4.6
3-Methylbenzoic acid	2.4	Fluoranthene	4.7
4-Chlorophenol	2.4	Dibenzyl	4.8
Trichloroethene	2.4	2,6-Diphenylpyridine	4.9
Atrazine	2.6	Triphenylamine	5.7
Ethyl benzoate	2.6	DDT	6.2
2,6-Dichlorobenzonitrile	2.6		

Plant-bacteria synergism for the phytoremediation of organics

After plant uptake, the organic compound may be metabolized and/or released into the atmosphere via evapotranspiration through the stem and/or leaves. Although plants often metabolize or sequester organics, they are at a significant disadvantage in two ways [19•]: (1) being photoautotrophic, plants do not rely on organic molecules as a source of energy or carbon. By consequence, unlike microorganisms, during evolution plants were not under selective pressure to develop the capacity to degrade chemically recalcitrant molecules, leading to a much more limited spectrum of chemical structures that they can metabolize; (2) to avoid build-up and potential toxicity to sensitive organelles, plant metabolism of organic molecules (other than photosynthates) consists of general transformations to more water-soluble forms, and sequestration processes (green-liver model: [19•]). By contrast, microbial metabolism often ends with the organics being converted into CO_2, water and cellular biomass.

Therefore, in order to obtain a more efficient degradation of organic compounds, plants depend on their associated microorganisms (Figure 2). Plants themselves have a positive effect on the microbial degradation of organic contaminants [20]. This increased degradation potential is the result of higher microbial densities and metabolic activities in the rhizosphere due to microbial growth on root exudates and cell debris originating from the plant roots. Moreover, dense populations of diverse heterotrophic microorganisms are living in the rhizosphere, the phyllosphere and inside the plant (endophytes). These microbial associations increase the capacity for a stepwise transformation of organic contaminants by consortia and provide a habitat that is conducive to genetic exchange and gene rearrangements. The emerging picture suggests that plants draw pollutants, including PAHs, into their rhizosphere to varying extents via the transpiration stream [21].

Figure 2



Contribution of plants and their associated bacteria to phytoremediation of organic contaminants.

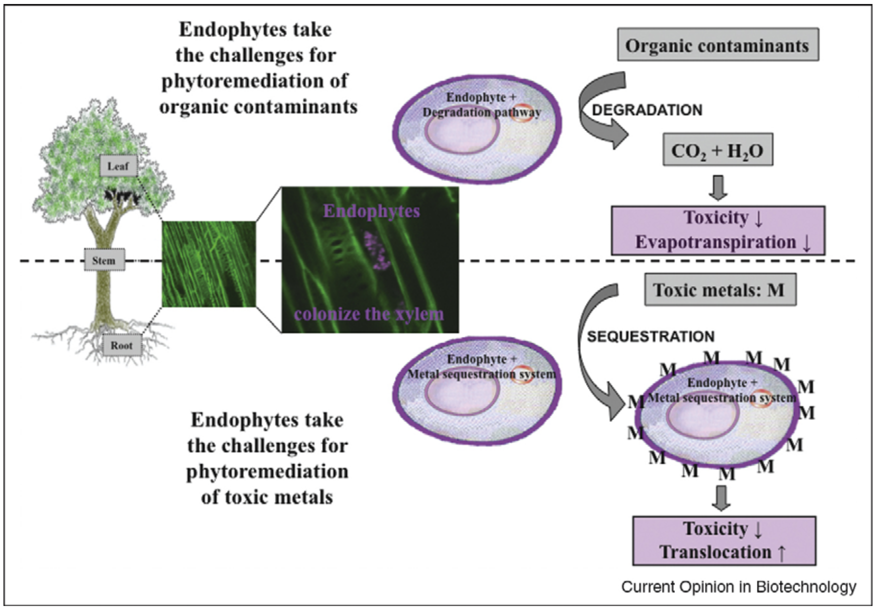
Subsequent degradation can occur in the plant itself, or in the rhizosphere, or both. However, compounds with a log K_{ow} between 0.5 and 3.5 seem to enter the xylem faster than the soil, and rhizosphere microflora can degrade them, even if the latter is enriched with bacteria capable of degrading the compound [22]. Therefore, after this class of compounds is taken up by the plants, endophytes seem to be especially suitable for the degradation of these compounds.

To conclude, it is obvious that plant–microbe partnerships are extremely valuable for a successful remediation of organic contaminants. The importance of plant–microbe partnerships in the remediation of organic contaminants was confirmed in studies at the level of the rhizosphere [23,24], the phyllosphere [25•] and inside the plant [12,26•,27••,28,29]. An overview of the use of plant-based technologies for the remediation of organic contaminants was provided in [30•]. Porteous Moore *et al.* [12] investigated the diversity of endophytic bacteria associated with hybrid poplar trees growing on a BTEX-contaminated site. They demonstrated that within the diverse bacterial communities found in poplar, several endophytic strains were capable of degrading BTEX-compounds. Furthermore, Barac *et al.* [28] demonstrated on the same site that after remediation, when the BTEX-concentration decreased below the detection limit, the degradation capacity of the endophytic community disappeared that brings us back to the original, natural situation. Recently, the diversity of the cultivable bacteria found in association with English oak and common ash, growing side by side on the same TCE-contaminated site, was investigated [50]. The majority of the isolated bacteria showed increased tolerance to TCE, and TCE degradation capacity was observed in some of the strains.

Endophytes take the challenge to improve phytoremediation of organics

Although successfully applied in several demonstration projects, large-scale field application of phytoremediation of organic pollutants is limited by several restrictions: (a) the levels of contaminants tolerated by the plant, (b) the often limited bioavailability of the contaminants and, (c) in certain cases, unacceptable levels of evapotranspiration of volatile organic contaminants to the atmosphere. A possible solution to conquer these constraints is the use of genetically manipulated plants specifically tailored for phytoremediation purposes [31••]. However, since bacteria are much easier to manipulate than plants, and natural gene transfer between closely related environmental and endophytic species is possible (avoiding the limitations of using GMOs), many studies have focussed on the use of natural or engineered plant-associated bacteria. The state of the art of rhizosphere 'engineering' for accelerated rhizodegradation of persistent organic contaminants was recently reviewed in [32]. Even when an efficient rhizodegradation seems possible, compounds with a lipophilicity in the optimum range seem to enter

Figure 3



Endophytes take the challenges for phytoremediation.

the root xylem before the soil and rhizosphere microflora can degrade them [22]. Since the residence time of contaminants in the xylem ranges from several hours to up to two days [33], (engineered) degrading endophytes colonizing the xylem are perfect candidates to reduce phytotoxicity and to avoid evapotranspiration of contaminants or their degradation intermediates into the environment (Figure 3). If no naturally occurring endophytes with the desired metabolic properties are available, endophytic bacteria can be isolated, equipped with the appropriate degradation pathways and subsequently re-inoculated in their host plant. The general idea behind the use of engineered endophytes to improve phytoremediation is to complement the metabolic properties of their host plant. Proof of this concept was provided by inoculating yellow lupine plants [27••] and poplar [29] with endophytic bacteria able to degrade toluene, which resulted in decreased toluene phytotoxicity and significantly lowered toluene evapotranspiration.

As many catabolic pathways are occurring in soil bacteria, where they are often encoded on self-transferable plasmids or transposons, natural gene transfer offers huge potential for the *a la carte* construction of endophytic bacteria with appropriate catabolic pathways. Heterologous expression of these catabolic functions might not constitute a major problem, especially when the donor and the recipient endophytic strains are closely related. Other applications than remediation can also be envisaged, such as protection of the food chain by reducing residual levels of agrochemicals in food crops. Recently, the use of bacterial endophytes for reducing levels of toxic herbicide residues in crop plants was successfully demonstrated [34•]. Inoculation of pea plants (*Pisum sativum*) with a poplar endophyte able to degrade 2,4 dichlorophenoxyacetic acid (2,4-D) resulted in an increased ability to remove 2,4-D from the soil, while the plants did not accumulate 2,4-D in the tissues nor showed toxic effects [34•].

Although it is obvious that the application of engineered plant-associated bacteria to improve phytoremediation of organic contaminants has high potential, some questions still need to be solved before large-scale field use [35•]. An important issue is the persistence and the stability of the engineered organisms and their degradation capabilities in association with plants growing in the field. As long as there is a selection pressure, there will be a selective advantage for those community members possessing the appropriate degradation characteristics [28]. Nevertheless, this is no guarantee that inoculated strains will become an integrated part of the endophytic community. However, instead of integrating a new strain, the endogenous microbial community can also get adapted through horizontal gene transfer. Horizontal gene transfer has been illustrated to perform an important role in the adaptation of microbial communities to environmental stress factors, including rhizospheric [36,37] and endophytic communities [29]. This may have the practical advantage that no long-term establishment of inoculants is required.

Endophytes take the challenge to improve phytoextraction of toxic metals

The weak points of metal phytoextraction are well recognized and its optimization still requires much research [38,39]. Metal availability, metal uptake and phytotoxicity for the plant are the main limiting factors for the application of phytoextraction. Phytoextraction is a long-term process, it may not be able to remove 100% of the contamination and, until now, its efficiency has only been demonstrated for some metals [40]. To optimize phytoextraction, genetic manipulation of plants [31••,41] as well as manipulation of the plant-associated microbial communities has been considered [42]. Possible manipulation strategies of the plant-associated community to improve the efficiency of phytoextraction include (a) isolation of associated bacteria, followed by equipping them with (a1) metabolic pathways for the synthesis of natural chelators, such as citric acid to improve metal availability for plant uptake and translocation and with (a2) metal sequestration systems to reduce phytotoxicity; and re-inoculation of these modified bacteria [7•,43•]; as well as (b) enrichment of bacteria present *in planta* [9,44–47] (Figure 3). For instance, *Lupinus luteus* L, when grown on a nickel enriched substrate and inoculated with the engineered nickel-resistant endophytic bacterium *B. cepacia* L.S.2.4::*ncc-nre*, showed a significant increase (30%) of nickel concentration in the roots, whereas the nickel concentration in the shoots remained comparable with that of the control plants [7•].

Phytoremediation of mixed waste pollution

Although there exists an obvious difference in phytoremediation potential whether organics or metals are the primary targets; at most contaminated sites, plants and their associated microorganisms will have to deal with mixed contamination. Remediation of these mixed waste sites is generally intricate. The occurrence of toxic metals potentially inhibits a broad range of microbial processes, including the degradation of organic pollutants [48•]. A very promising strategy to tackle the mixed waste situation is the use of endophytes that are capable of (a) degrading organic contaminants and of (b) dealing with, or in the ideal scenario, accelerating the extraction of toxic metals. It has been shown that engineering of rhizobacteria for TCE degradation and heavy metal (Cd) accumulation resulted in an increased Cd accumulation but also in a lowered toxic effect of Cd on the TCE degradation [49]. Similar improvements are expected when these engineered rhizobacteria are inoculated onto plant roots.

Conclusions

The exploitation of plant–endophyte partnerships for the remediation of contaminated soils and (ground) water is a promising area. For example, endophytes can be developed to promote sustainable production of bioenergy crops in conjunction with phytoremediation of contaminated soils and (ground) water, or to improve revegetation and sustainable feedstock production on marginal land in general [14].

In the case of phytoremediation of organic contaminants, endophytic bacteria possessing the appropriate degradation pathway(s) can assist their host plant by degrading contaminants that are readily taken up by plants, which fail to degrade them to completion, resulting in (a) toxicity owing to the accumulation of the original compound and/or degradation intermediates or (b) evapotranspiration of volatile contaminants. In the case of phytoremediation of toxic metals, endophytes equipped with a metal-sequestration system and/or able to produce natural metal chelators can reduce metal toxicity for their host plant and/or increase metal translocation to the aerial parts.

Furthermore, to tackle co-contaminated soils or (ground) water, (engineered) endophytes that are capable (a) to degrade the organic contaminants, and (b) to deal with, or even to improve extraction of the toxic metals, can be used.

In order to further optimize endophyte stimulated phytoremediation, plant–endophyte interactions should be studied in more detail. With the availability of complete genome sequences for many plant-associated bacteria, our information-base concerning plant-associated bacteria, including phytopathogens and phytosymbionts, is exponentially growing. To maximally exploit these data, high throughput approaches are required, in which all or most of the genes, proteins, or even metabolites in an organism are subjected to functional analysis.

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