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Research review paper

Using soil bacteria to facilitate phytoremediation

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ABSTRACT

In the past twenty years or so, researchers have endeavored to utilize plants to facilitate the removal of both organic and inorganic contaminants from the environment, especially from soil. These phytoremediation approaches have come a long way in a short time. However, the majority of this work has been done under more controlled laboratory conditions and not in the field. As an adjunct to various phytoremediation strategies and as part of an effort to make this technology more efficacious, a number of scientists have begun to explore the possibility of using various soil bacteria together with plants. These bacteria include biodegradative bacteria, plant growth-promoting bacteria and bacteria that facilitate phytoremediation by other means. An overview of bacterially assisted phytoremediation is provided here for both organic and metallic contaminants, with the intent of providing some insight into how these bacteria aid phytoremediation so that future field studies might be facilitated.

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1. Introduction

The population of planet Earth will reach the 7 billion mark around the beginning of 2010 and, at the current rate of increase, it is estimated that it will be around 8 billion by 2019 (http://www.ibiblio.org/lunarbin/worldpop). Moreover, around "40% of deaths worldwide are caused by water, air and soil pollution" and "such environmental degradation, coupled with the growth in world population, are (considered to be) major causes behind the rapid (global) increase in human disease" (http://www.sciencedaily.com/releases/2007/08/070813162438.htm). Thus, as a consequence of both increasing population and industrial technology, humanity has created a situation where many life forms, including humans, are increasingly at risk. That is, until relatively recently, it was generally believed that earth's atmospheric, terrestrial, and aquatic systems were sufficient to absorb and break down wastes from population centers, industry, and agriculture. Unfortunately, we now know that this is not true. Thus, notwithstanding recent global efforts to curb human activities that are detrimental to the environment and human health, we are faced with a world that is highly contaminated with a range of toxic metals and organic compounds.

The problem of toxic waste disposal is enormous. For example, it was estimated that in 1993 approximately 275 million tons of hazardous waste was produced in the United States (Ziegler, 1993). Moreover, in 1996, in the United States the Environmental Protection Agency listed 39,925 sites on its inventory of uncontrolled waste sites (DeRosa et al., 1996). Of the many chemicals found in hazardous waste sites in the

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United States, the Agency for Toxic Substances and Disease has created a list of the 275 hazardous substances that pose the greatest threat to human health (http://www.atsdr.cdc.gov/cercla/07list.html). The top ten "priority substances" are given in Table 1.

To avoid the toxicity associated with these hazardous compounds, several technologies and methods have been developed to remove them from polluted soils. Many of these methods include the physical removal of soil to landfill sites or extraction through chemical or physical means. These techniques are rapid but, unfortunately, they are costly from both an economic and an environmental point of view, and could potentially have a deleterious impact on soil physical, chemical, and biological properties. In addition, the removal from the environment of many toxic compounds is complicated by the numerous classes and types of these chemicals. For example, many soils are contaminated with one or more metals, other inorganic compounds, radioactive material or various organic compounds. Of these, the metals may include lead, zinc, cadmium, selenium, chromium, cobalt, copper, nickel or mercury; the other inorganic compounds might include arsenic, sodium, nitrate, ammonia or phosphate; the radioactive compounds may be uranium, cesium or strontium; and the organic compounds may include chlorinated solvents like trichloroethylene; explosives such as trinitrotoluene (TNT) and 1,3,5-trinitro-1,3,5-hexahydrotriazine (RDX); petroleum hydrocarbons including benzene, toluene and xylene (BTX), polycyclic aromatic hydrocarbons (PAHs); and pesticides such as atrazine and bentazon.

2. Phytoremediation

While some organic compounds can be metabolized (i.e., remediated) by bacteria that may either be found in or added to the soil, in the absence of plants, this process is usually slow and inefficient, in part as a consequence of the relatively low numbers of these degradative microorganisms in soil (Brookes and McGrath, 1984). On the other hand, the use of plants to remediate polluted soils (i.e., phytoremediation; Salt et al., 1995) is a potentially clean, effective and relative inexpensive technology that is likely to be readily accepted by a concerned public. However, this approach also has its drawbacks, as few plant species can naturally tolerate and/or accumulate high concentrations of the above mentioned environmental contaminants.

Some organic compounds can be directly degraded and completely mineralized by plant enzymes through phytodegradation (Alkorta and Garbisu, 2001; Wild et al., 2005); many plants produce, and often secrete to the environment, enzymes that can degrade a wide range of organic compounds. However, inorganic pollutants cannot be degraded. Inorganic pollutants must either be stabilized in the soil to make them less bioavailable (i.e., phytostabilization); extracted, transported, and accumulated in plant tissues (i.e., phytoextraction); or transformed into volatile forms (i.e., phytovolatilization) (Pilon-Smits, 2005). Phytoremediation efficiency for metals is often limited by the bioavailability of the metal in soil, plant root development, and the level of tolerance of the plant to each particular metal (Pilon-Smits, 2005).

Table 1
U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry for 2007 (http://www.atsdr.cdc.gov/cercla/07list.html).

Rank	Substance
1	Arsenic
2	Lead
3	Mercury
4	Vinyl chloride
5	Polychlorinated biphenyls
6	Benzene
7	Cadmium
8	Polycyclic aromatic hydrocarbons
9	Benzo(A)Pyrene
10	Benzo(B)Fluoranthene

Unfortunately, many of the plants that are most effective at removing metals from the soil, i.e., hyperaccumulators such as *Thlaspi caerulescens* (Alpine pennycress) and *Alyssum bertolonii*, are small and slow growing, thus reducing their potential for metal phytoextraction from soil on a large scale (Khan et al., 2000). To be effective for the remediation of metal polluted soils, plants must be tolerant to one or more metals, highly competitive, fast growing, and produce a high aboveground biomass. Because of their high biomass and extensive root system, trees are considered to be attractive for phytoremediation; however, metal accumulation by trees is generally low.

Metal phytoextraction (as well as plant growth) can sometimes be facilitated by soil microorganisms living in intimate association with plant roots (Shilev et al., 2001). In addition, the biodegradation of recalcitrant organic compounds in the soil is often enhanced around the roots of plants. Following root exudation, the proliferation of specific groups of microorganisms, able to aggressively colonize the root surface and affect plant growth, occurs (Kloepper et al., 1989). As a consequence of the high level of nutrients that plants release into the soil as root exudates, the concentration of bacteria in the immediate vicinity of plant roots (i.e. the rhizosphere) is typically 10- to 1000-fold greater than the bacterial concentration that is found in the bulk soil. Some rhizosphere microorganisms can act directly on organic pollutants using their own degradative capabilities (phytostimulation or rhizodegradation) (Kuiper et al., 2004). As well, some soil bacteria can positively affect plants by improving growth and health (Glick, 1995), enhancing root development (Gamalero et al., 2002, 2004), or increasing plant tolerance to various environmental stresses (Glick, 2004). In turn, larger and healthier plants are better able to phytoremediate both organic and inorganic contaminants. This review provides an overview of the involvement of soil bacteria in "assisted phytoremediation". Since various aspects of the work described here have previously been reviewed, from several different perspectives (Glick, 2003; Khan, 2005; Newman and Reynolds, 2005; Pilon-Smits, 2005; Krämer, 2005; Pilon-Smits and Freeman, 2006; Arshad et al., 2007; Jing et al., 2007; Zhuang et al., 2007; Doty, 2008; Kamaludeen and Ramasamy, 2008; Reichenauer and Germida, 2008; Yang et al., 2009; Gamalero et al., 2009; Gerhardt et al., 2009; Rajkumar et al., 2009; Weyens et al., 2009), this article is directed toward developing an understanding of the underlying principles of bacterially assisted phytoremediation as elaborated in the more recent scientific literature.

3. Phytoremediation of organics facilitated by bacteria

Bacteria may interact with and affect the growth of plants in a variety of ways. Some bacteria are phytopathogenic and actively inhibit plant growth; others (plant growth-promoting bacteria) can facilitate the growth of plants using a wide range of different mechanisms; and there are a large number of soil bacteria that do not appear to affect the growth of plants one way or the other, although this may vary as a function of a range of different soil conditions (Glick, 1995).

3.1. Biodegradative bacteria

It has been known for some time that many soil bacteria are able to degrade toxic organic compounds (Chakrabarty, 1981). With the discovery of a number of soil microorganisms that are capable of degrading xenobiotic chemicals including herbicides, pesticides, refrigerants, solvents, and other organic compounds, the notion that microbial degradation might provide a reasonable and effective means of disposing of toxic chemical wastes gained credence.

Pseudomonas spp. are the most predominant group of soil microorganisms that biodegrade complex organic compounds, a process that typically requires the concerted efforts of several different enzymes. The genes that code for the enzymes of these biodegradative pathways are often located on large (~50 kb to 200 kb) plasmids (Ghosal et al., 1985; Cork and Krueger, 1991).