Korean J. Soil Sci. Fert. 44(1), 58-66 (2011) **Article**

Exploring the Potential of Bacteria-Assisted Phytoremediation of Arsenic-Contaminated Soils

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Arsenic pollution is a serious global concern which affects all life forms. Being a toxic metalloid, the continued search for appropriate technologies for its remediation is needed. Phytoremediation, the use of green plants, is not only a low cost but also an environmentally friendly approach for metal uptake and stabilization. However, its application is limited by slow plant growth which is further aggravated by the phytotoxic effect of the pollutant. Attempts to address these constraints were done by exploiting plant-microbe interactions which offers more advantages for phytoremediation. Several bacterial mechanisms that can increase the efficiency of phytoremediation of As are nitrogen fixation, phosphate solubilization, siderophore production, ACC deaminase activity and growth regulator production. Many have been reported for other metals, but few for arsenic. This mini-review attempts to present what has been done so far in exploring plants and their rhizosphere microbiota and some genetic manipulations to increase the efficiency of arsenic soil phytoremediation.

Key words: Arsenic pollution, Phytoremediation, Rhizosphere bacteria, Plant-microbe interaction

Introduction

Arsenic (As) contamination from geogenic and anthropogenic sources has occurred in many parts of the world and is now recognized as a global problem (Nriagu, et al., 2007). Arsenic was categorized as the most hazardous chemical by the US Department of Health (ATSDR, 2005). Due to its toxicity, As poses a serious threat to human health. Although, it was used as early as 2500 years ago to the early 20th century for medicinal purposes, exposure to this poison results in arsenicism, hyperpigmentation, keratosis, cancers especially skin cancer, gastro-intestinal disturbances, respiratory and pulmonary diseases, cardiovascular abnormalities and diseases, hepatic diseases and hematological effects (Mandal and Suzuki, 2002). The main pathway of As exposure of humans is through ingestion of As contaminated water, consumption of foods and, to a lesser extent, inhalation of contaminated air (Nriagu et al., 2007). Great attention has been given to As recently as millions have suffered from As poisoning due to drinking As-contaminated water extracted from shallow tube wells in South and South East Asia (Nordstrom, 2002) especially in Bangladesh and West Bengal, India.

Being a serious environmental problem, technologies have been implemented for the remediation of As. The remediation of As requires a specific approach since like metals and other metalloids it cannot be degraded and hence requires appropriate methods for their removal (Rajkumar et al., 2009). Remediation technologies for heavy metals have been employed from physical and chemical techniques which are not only costly but also compromise the soil physical, biological and chemical properties (Pulford and Watson, 2003). An alternative technology is bioremediation which relies on microbial activity to reduce, mobilize, or immobilize As through sorption, biomethylation, complexation, and oxidation-reduction processes (Wang and Zhao, 2009). Another technology that is gaining ground in recent years is

Received : January 20. 2011 Accepted : February 22. 2011
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phytoremediation. The discovery of plants that can take up heavy metals in large amounts created optimism for the remediation of polluted lands. Furthermore, the biotechnological use of microorganisms in association with plants offers more advantages for heavy metal uptake or removal (Zhuang et al., 2007). Several mechanisms were identified by which the interaction of plants and microbes especially rhizosphere microbes enhance remediation of polluted soils and were reviewed extensively elsewhere (Glick, 2010; Ma et al., 2011). It is the aim of this review to present what has been done in increasing the efficiency of phytoremediation especially the use of bacteria in assisting phytoremediation of As.

Arsenic Contamination in the Soil

Arsenic can be found in nature in virtually all environmental media. It is widely distributed in the Earth's crust though in low abundance (0.0001%) (Nriagu, 2002). Geochemical sources of As compounds include As-rich parent material as As easily substitutes for Si, Al, or Fe in silicate minerals (Bhumbla and Keefer, 1994). Other natural sources of As are volcanic activities (O'Neill, 1995), windblown soil particles, sea salt sprays and microbial volatilization of As (Nriagu et al., 2007). The mean estimated global atmospheric emission of As from natural sources is about 12 Mt (Nriagu, 1989).

The levels of As in the soils of various countries are estimated to range from 0.1 to 40 mg kg^{-1} (mean 6 mg kg^{-1}), 1 to 50 mg kg^{-1} (mean 6 mg kg^{-1}) and mean 5 mg kg^{-1}. Arsenic contents of Japanese paddy soils were found to be higher than those found in Korean soils, 10.3 ± 8.5 mg kg^{-1} and 4.6 ± 2 mg kg^{-1}, respectively (Yang et al., 1999). The difference is possibly due to the difference in parent material, granite in Korea and volcanic ash in Japan (Yang et al. 1999).

In addition to the As that occurs naturally, Nriagu et al (2007) reported that over 80% of all the As ever produced by man have dissipated to the environment. The global annual As emissions from anthropogenic sources is estimated to be 19 kt to the atmosphere, 82 kt in the soil and 42 kt in aquatic environments (Nriagu and Pacyna, 1988). The major anthropogenic contributors of As are mining, smelting and ore processing, pesticides, fertilizers, and chemical industries, thermal power plants using coal or peat, wood preservation industries using chromated copper arsenate (CCA) and incinerations of preserved wood wastes (Pacyna and Pacyna, 2001). Arsenic has also been used in chemical warfare agents (Nriagu et al., 2007).

In agriculture, inorganic compounds of arsenic have been widely used in pigments, insecticides, herbicides and fungicides for a century. Because of its phytotoxicity it was used as herbicides, desiccant to cotton and for defoliation of seed potatoes. In the form of lead arsenate it was used to control insect pests. Arsenic is also used as an additive in chicken feeds (O'Neill, 1995), roxarsone-4-hydroxy-3-nitrobenzene arsonic acid being the most common As-based additive. The practice of using As-based ingredients in the poultry industry started in the 1970's. Furthermore, the widespread use of chicken manure as fertilizer has contributed to increased As levels in soil and especially in groundwater (Rutherford et al., 2003). Irrigation with contaminated groundwater further increases accumulation of As in soils as is the case in Bangladesh (Nriagu et al., 2007). The use of CCA and other As-based chemicals in wood preservation industries has also caused widespread contamination of soils and aquatic environments (Bhattacharya et al., 2002).

In Korea, sources of trace metal causing soil and groundwater contamination are derived directly or indirectly from mining sites, industrial or domestic wastewater, solid wastes, and sewage sludge (Yang et al., 1999). The smelter industry and the combustion of fossil fuels constitute a considerable amount of trace metal in the soil and groundwater (Kim, 1993). Along with Cd, Cr, Cu, Hg, Pb and Zn, As is a major concern in Korea due to its phytotoxicity (Yang et al., 1999). The major industries that contribute to As pollution are mining and agrochemicals and the major pollution pathway is through irrigation water (Kim, 1989).

In nature, As exists in four oxidation states: (-III), (0), (+III) and (+V). With different physical and chemical properties, the various chemical forms of As available are arsenate (As V), arsenite (As III), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine (TMA), arsenocholine (AsC), arsenobetaine (AsB) and arsenosugars. Although analogous to P, As is not directly available to plants. The inorganic arsenate and arsenite are the main phytoavailable forms of As in soil solution (Meharg and Hartley-Whitaker, 2002). Arsenate and arsenite usually dominate in As-contaminated soil. In aerobic soils, arsenate is predominant (Smith et al., 2010) while arsenite are predominant in paddy soils (Panda et al., 2010).

Arsenic Remediation

Due to the toxicity of As, technologies have been employed for its remediation. Remediation refers to the processes or methods for treating contaminants in soil or water such that they are contained, removed, degraded, or rendered less harmful. There are several subcategories of remediation. *In situ remediation* refers to treatment or stabilization of soil or water contaminants in place, whereas *ex situ remediation* involves physical removal and treatment of either soil or water at another location (Pierzynski et al., 2005). Soil contaminated with As has traditionally been addressed with conventional cleanup technologies such as removal (excavation and landfilling) and containment (capping). Due to the high cost of removal (Table 1), and loss of land use by containment, cost-effective *in situ* remedies are being explored.

Although it has been employed for decades, bioremediation is still considered by the US Environment Protection Agency as an innovative technique (Atlas and Philip, 2005). It is an *in situ* cost-effective technology in contaminant removal and with the current development of bioengineering and greater understanding of microbial diversity; it can greatly increase efficiency in the restoration of the environment. Most bioremediation techniques have been employed for remediation of oil spills and organic contaminants, however, at present, no commercial bioremediation technology is available for soils contaminated with As.

Table 1. Typical costs of land remediation techniques (Atlas and Philip, 2005).

Remediation technique	Cost ($ US per m^3)
Thermal treatment (on-site incineration)	178-715
Excavation and disposal	53-134
Soil washing	26-71
Engineering capping	26-62
Encapsulation with geomembranes	71-107
Solidification/stabilization	17-178
In-situ chemical oxidation	71-152
Bioremediation	2-268

Phytoremediation

Phytoremediation is another new concept where living plants are directly used for *in situ* remediation of contaminated soil, sludges, sediments, and ground water through contaminant removal, degradation, or containment (US EPA, 1999). It is an attractive technology for remediation of contaminated soils due to its low cost and aesthetic advantages (Nriargu et al., 2007).

Different technologies of phytoremediation applicable for arsenic are summarized in Table 2.

To be effective for remediation, there are some considerations for the choice of plants. These have to be tolerant, highly competitive, fast growing, and producing high aboveground biomass (Glick, 2010). A variety of tolerance and resistance mechanisms enable various plants to protect themselves from the toxic effects of metals. Generally, plants avoid or exclude metals from accumulating in their cells. However, some plants survive while accumulating high concentrations of metals. Root exudation of organic ligands is considered as one of the most important strategies by which plants exclude metals such as Al, Cd and Pb. These mechanisms enable metal tolerating species to restrict uptake and translocation of metals, maintaining a low shoot metal concentration (Kidd et al., 2009). Cai and Ma (2003) gave an extensive review of the several mechanisms involved in metal tolerance by plants which includes chelation, intercellular partitioning especially to the vacuole, and the possible alterations of cellular metabolism such as enzymes. Alterations may involve the membrane structures but this has only been demonstrated in copper (Cai and Ma, 2003).

Different crop species have different sensitivities and tolerance to As. Although generally, beans are sensitive to metals, some leguminous plants have the capacity to tolerate metals. *Lupinus albus* was found to be a good candidate for stabilizing As and Cd in soils (Vazquez et al., 2006). Several benefits in using this legume include improvement in soil properties due to atmospheric N fixations, increase in the pH of acidic soils, decrease in $CaCl_2$-extractable As and Cd, and retention of these elements in the roots. On the other hand, graminaceous plants that are capable of producing phytosiderophores can efficiently chelate ferric iron due to their amine and

Table 2. Phytoremediation technologies applicable for arsenic (Salt et al., 1995; Wenzel et al., 1999).

Technology	Description
Phytostabilization	The containment process using plants and is often used in combination with soil additives to mechanically stabilize the site and reduce pollutant transfer to other ecosystem compartments and the food chain
Phytoextraction	The removal process of pollutant by taking advantage of the ability of some plants to hyperaccumulate metals into their shoots
Phytovolatilization	The removal of pollutants by employing metabolic processes by the plant and their associated rhizosphere microorganisms and transform the pollutants into volatile compounds.

Table 3. Arsenic concentrations in hyperaccumulator and As tolerant plants (Fitz and Wenzel, 2002; Zhao et al., 2002).

Plant species	As in plants (Fronds/Shoot)	Reference
	mg kg^{-1}	
Hyperaccumulators		
Pteris vittata	755-22,630	Ma et al., 2001
P. cretica	2,000-2,800	Zhao et al., 2002
P. longifolia	5,000	Zhao et al., 2002
P. umbrosa	5,000	Zhao et al., 2002
Pityrogramma calomelanos	8,000	Francesconi et al., 2002
Tolerant plants (non-accumulators)		
Agrotis capillaries	3470	Porter and Peterson, 1975
Agrotis catellana	170	De Koe, 1994
Agrotis delicatula	300	De Koe, 1994
Cynodon dactylon	1,600	Jonnalagadda and Nenzou, 1997
Paspalum tuberosum	1,130	Bech et al., 1997
Spegulania grandis	1,175	Bech et al., 1997

carboxyl groups. In Fe deficient soils, these plants can significantly increase the release of phytosiderophores which are capable of solubilizing not only Fe but also Mn, Cu, Zn, Hg, phosphate and arsenate in the rhizosphere (Treeby et al., 1989; Meagher and Heaton, 2005). Thus, co-cultivation of grasses has been suggested for potential remediation of low-level contaminated soils (Luo et al., 2008).

It has been recognized earlier that some plants growing on metalliferous areas can accumulate large amounts of heavy metals on their above ground biomass (Baker, 1981). Thus, their potential for phytoremediation has created much interest in both academic research and practical applications. Plants that absorb exceptionally high amounts of metals are termed as hyperaccumulators. The first reported As hyperaccumulator was *Pteris vittata* or Chinese brake fern (Ma et al., 2001). Several other fern species were then investigated for their hyperaccumulation ability. *Pityrogramma calomelanos* (Francesconi et al., 2002) and other species of the *Pteris* family were also reported to hyperaccumulate As in varying degrees (Table 3). Studies on *P. vittata* and other fern species and effects of soil amendments, other metals and other factors influencing hyperaccumulation of these plants were reviewed by Butcher (2009).

Plants that do not accumulate but can tolerate large amounts of As can be potentially used for phytostabilization. A 5-year study by King et al. (2008) using four *Eucalyptus* spp. found that *E. cladocalyx* had the highest biomass and did not to affect As availability making it a good candidate for phytostabilization of As in gold mine tailings. In an earlier study, Vazquez et al. (2006) reported that white lupin reduced Cd and As solubility in the soil while it accumulated significant amounts of the metals in its roots and nodules.

Phytoremediation has its limitations such as few plant species can tolerate or accumulate high levels of metals in their tissues and these plants are small and slow growing.

Bacteria-Assisted Phytoremediation

Microbial-plant interactions have been greatly studied during the last 50 years; however, these were primarily focused on plant-pathogen interactions. It was only recently that investigations of microbial interactions in the rhizosphere were aimed at decontamination processes (Kavamura and Esposito, 2010).

The root zone is the site where intensive interactions take place between plants, soil and soil microorganisms. Plants release from the roots large amounts of low molecular weight water soluble exudates such as amino acids, hormones, organic acids, sugars and vitamins (Antoun and Kloepper, 2001) which become source of nutrition of microorganisms in the rhizosphere. The presence of these microorganisms in the rhizosphere can have a neutral, deleterious or beneficial effect on plant growth. Bacteria that aggressively colonize plant roots which have beneficial effect on plant growth are referred to as plant growth promoting rhizobacteria (PGPR) and many of these have been extensively studied for biofertilizer applications. Recently, this group of bacteria has also been explored for use in stressed environments such as saline and flooded environments (Saleem et al., 2007). PGPR can promote plant growth through direct and indirect mechanisms. These mechanisms include phosphate solubilization, nitrogen fixation, reduction of ethylene production through the action of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Reed and Glick, 2005; Safronova et al., 2006; Saleem et al., 2007), siderophore production and production of hormones such as cytokinins and auxins. These mechanisms can also benefit plants grown in heavy metal contaminated soils. Heavy metals and metalloids are known to cause stress on plants, thereby reducing growth and viability of plants. ACC deaminase containing bacteria can cleave ACC, the precursor of ethylene, to ammonia and α-ketobutyrate, thus, reducing stress ethylene levels and promoting plant growth even in contaminated soils (Gerhardt et al., 2006). Siderophore producing bacteria, on the other hand, could be of particular importance in heavy metal contaminated soils. These bacteria can provide iron to plants which can reduce the phytotoxic effects of metals (Dimpka et al., 2008; Sinha and Mukherjee, 2008). Moreover, siderophores produced by soil microbes play an important role in complexing toxic metals and in increasing their mobility in soils (Rajkumar et al., 2010).

Isolation of bacteria from plant rhizospheres are usually conducted with the prospect of using these isolated PGPRs in phytoremediation of As contaminated soils. Several bacterial strains associated with the roots of *Cirsium arvense* (L.) growing in an As contaminated soil in Italy were screened for tolerance and plant growth promoting traits as initial steps in increasing the efficiency of phytoremediation (Cavalca et al., 2010b). Of the 64 As-resistant strains, most have at least one PGP trait. Only 3 of the isolated strains were able to produce IAA and siderophore and were positive for ACC deaminase activity. The authors concluded that As-resistant bacteria which possess various PGP traits can potentially support plant growth in As-polluted soil and reduce stress symptoms. In another study, it was reported that *Alcaligenes* sp. DhalL colonized sunflower rhizosphere and promoted As uptake of plants (Cavalca et al. 2010a). Although biomass of sunflower plants was not significantly enhanced, inoculation of *Alcaligenes* sp. DhalL significantly increased As accumulation in plant tissues. In a greenhouse experiment, bacteria and fungi isolated from the rhizospheres of Kikuyu grass and Rainbow fern growing in As-contaminated cattle dip sites promoted As accumulation by 45% when inoculated in the grass *Agrotis tenius*. Among the identified resistant bacteria were *Arthrobacter* sp. and *Bacillus* spp. (Chopra et al., 2007). The most commonly studied groups of bacteria for phytoremediation are free-living. However, some symbiotic bacteria were also found to be possible candidates in assisting phytoremediation. A resistant *Rhizobium* strain isolated from nodules of *Vigna mungo* (L.) Hepper (Mandal et al., 2008) can be further studied for its use in environmental restoration and improving plant growth. Another rhizobial strain, *Bradyrhizobium japonicum* strain CB1809 was found to increase biomass of soybean when grown in the presence of high concentration As compared to control (Reichman, 2007). Rhizobacteria also play an important role in the uptake and hyperaccumulation processes of *P. vittata* (Xiong et al., 2010). Although, As greatly affected the microbial community functional structure, the rhizosphere of *P. vittata* appeared to have mitigated the As toxicity to the microbes and maintained high-diversity of microbial species which most probably aided the hyperaccumulation of *P. vittata*.

Bacteria that colonize internal tissues of plants without causing negative effects on their hosts referred to as endophytic bacteria (Schulz and Boyle, 2006) also has potential to enhance phytoremediation. In fact, the beneficial effects of endophytic bacteria are generally greater than those of many rhizobacteria (Pillay and Nowak, 1997) especially when plants are growing under stress conditions (Barka et al., 2006). These endophytic bacteria may confer tolerance to plants under metal stress and may stimulate host plant growth through biological control, induced systemic resistance to pathogens, nitrogen fixation, production of growth regulators and enhancement of mineral nutrients and water uptake (Ryan et al., 2008). Other benefits include some physiological changes such as accumulation of osmolytes and osmotic adjustment, stomatal regulation, reduced membrane potentials and changes in phospholipid content in the cell membranes (Compant et al., 2005). Endophytic bacteria isolated from the Ni hyperaccumulator *Thlaspi goesingense* was found to tolerate high levels of the metal. However, no reports were found with endophytic bacteria specifically in relation to As phytoremediation. The inclusion of endophytic bacteria in the strategy of enhancing phytoremediation efforts for As contaminated soils is an area needed to be further explored.

Genetic transformations for phytoremediation have also been investigated. Transgenic canola expressing a bacterial ACC deaminase have increased germination rate compared to non-transformed canola when grown in the presence of arsenate. The mechanism implicated here was the reduction of stress-induced ethylene by the action of the ACC deaminase (Nie et al., 2002). Furthermore, biomass of canola was highest in transgenic canola inoculated with the PGPR *Enterobacter cloacae* CAL2 thus having highest arsenate accumulated. Sizova et al. (2006) reported the effect of inoculation of genetically modified *Pseudomonas aureofaciens* on the survival and As accumulation of sorghum. Three genes for arsenic tolerance, *arsR* (the transcription regulator), *arsB* (encoding a membrane protein) and *arsC* (encoding arsenate reductase) and *gltA* (citrate synthase, the gene for phosphate solubilization) were inserted to *P. aureofaciens*. Inoculation with citrate synthase modified *P. aureofaciens* resulted in higher survival and As accumulation of sorghum than with *arsRBC*-modified bacteria inoculated plants while wild strain inoculated plants all died. Using the model plant *Arabidopsis thaliana*, Dhankher et al. (2002) developed a strategy that increased shoot weight and As accumulation. Two bacterial genes, *arsC* and *γ-ECS* (*γ*-glutamyl synthetase) were co-expressed in *A. thaliana* and resulted in four-fold increase in shoot weight and 17- fold increase in As accumulation compared when only one of the genes was expressed. The authors mentioned that this strategy can be applicable to a wide variety of plant species. Bacterial *γ-ECS* was also inserted into eastern cottonwood and enhanced arsenate resistance was observed (Merkle, 2005). Field applications of these transgenics in phytoremediation are still very limited.

Conclusion

The use of plants and their associated bacteria for As soil remediation offers more advantages over the conventional methods employed. Phytoextraction and phytostabilization are two options for phytoremediation of As-contaminated soils. The discovery of *P. vittata* and other fern species was a breakthrough in As phytoremediation research. Commercialization of this technology has started in some areas in the United States and possibly in Asia. However, *P. vittata* or the other hyperaccumulating fern species are not always adapted in places where remediation is needed. Therefore, the continuous search for appropriate plants and their rhizosphere bacterial community especially those that promote growth and health of plants is needed. Moreover, a better understanding of the different mechanisms and factors involved in increasing the efficiency of bacteria-assisted phytoremediation is also needed for a more successful practical application of this technology for As clean up.

Acknowledgment

This work was partially supported by the Rural Development Administration (RDA), Republic of Korea.

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