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Simultaneous bioremediation of Cr(VI) and lindane in soil by actinobacteria

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ABSTRACT

Environments co-contaminated with metals and organic compounds are difficult to remediate. Actinobacteria is an important group of microorganisms found in soils, with high metabolic versatility and potential for bioremediation. In this paper, actinobacteria were used to remediate soil co-contaminated with Cr(VI) and lindane. Five actinobacteria, tolerant to Cr(VI) and lindane mixture were selected: *Streptomyces* spp. A5, A11, M7, and MC1, and *Amycolatopsis tucumanensis* DSM 45259. Sterilized soil samples were inoculated with actinobacteria strains, either individually or as a consortium, and contaminated with Cr(VI) and lindane, either immediately or after 7 days of growth, and incubated at 30 °C during 14 days. All actinobacteria were able to grow and remove both contaminants, the consortium formed by *Streptomyces* spp. A5, M7, MC1, and *A. tucumanensis* showed the highest Cr(VI) removal, while *Streptomyces* sp. M7 produced the maximum lindane removal. In non-sterile soil samples, *Streptomyces* sp. M7 and the consortium removed more than 40% of the lindane, while *Streptomyces* sp. M7 demonstrated the greatest Cr(VI) removal. The most appropriate strategy for bioremediation of Cr(VI) and lindane co-contaminated soils would be the inoculation with *Streptomyces* sp. M7.

1. Introduction

The great expansion of human activity caused by industrial growth has resulted in an increase in scenarios of serious and complex environmental contamination by both organic compounds (herbicides, plastics, tannins, polyphenols, pesticides, etc.) and inorganic compounds (As, Cd, Cu, Pb, Cr, Hg, etc.) (Volke Sepúlveda and Velasco Trejo, 2002). Mixed pollution caused by simultaneous contamination by organic and inorganic compounds is a widespread global problem that tends to be concentrated in certain types of locations such as industrial zones, oil storage areas, waste dumps, waste recycling sites, and soils and sediments near roads and railways (Volke Sepúlveda and Velasco Trejo, 2002). Co-pollution is a very important issue because more than one third of contaminated sites are found to have more than one type of pollutant (Tang et al., 2010; Mansour, 2012). Environments co-contaminated with metals and organic compounds are considered difficult to remediate because of the mixed nature of these pollutants.

Cr(VI) is a harmful pollutant characterized by its chronic toxicity, neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity and immunotoxicity (Bagchi et al., 2002), and Cr(VI) compounds are approximately 1000 times more toxic and mutagenic than Cr(III) compounds (USEPA, 1998; Dana Devi et al., 2001). However, Cr(VI) compounds have several uses in industry (Polti et al., 2007; Bhadra and Mahananda, 2013) and chromium contamination by these compounds in soil and water has been detected in and around a wide variety of industrial sites (Benimeli et al., 2003; Nie et al., 2010; Srinivasa Gowd et al., 2010).

The systematic use of pesticides has led to great improvements in terms of agricultural production levels. However, massive and indiscriminate application of pesticide products has also led to adverse effects on human health, the environment, and even the effectiveness of the products themselves (Johri et al., 2000; Phillips et al., 2005). The gamma isomer of hexachlorocyclohexane (γ-HCH), commercially known as lindane, is a highly chlorinated, recalcitrant organochlorine pesticide (OP). Lindane residues persist in the environment and have been reported in soils, water, air, plants, agricultural products, animals, foods, and microbial

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environments, as well as in the human body. Since the toxicity of γ-HCH is well established, it is now imperative to develop methods to remove lindane from the contaminated environments (Fuentes et al., 2011).

In recent years, the intense search for a solution to co-contamination has led to the development of remediation technologies that can address treatment of not just a single compound, but which can simultaneously deal with multiple contaminants (Srivastava et al., 2007; Ma et al., 2010; Wasi et al., 2011). However, studies involving methodologies for simultaneous decontamination of organic and inorganic contaminants remain rare, and have generally been focused on increasing organic biodegradation by reducing the toxicity of metals through their sequestration and precipitation. Such strategies are primarily concerned with degrading the organic contaminants without consideration of metal extraction, and consequently metal remediation is restricted to deactivation. It still remains imperative to develop low-cost removal techniques that can degrade organic compounds while also extracting metals or stabilizing them in a non-toxic or less toxic form (Puzon et al., 2002).

In the last ten years a stronger emphasis has come to be placed on the study of the physiological, biochemical, and molecular approaches to microbial bioremediation of environments co-contaminated with heavy metals and pesticides. Soils with long-term exposure to mixed contamination with organic compounds and heavy metals have been shown to have structural and functional microbial communities with the ability to adapt and grow under these conditions. This suggests that bioremediation based on microorganisms is feasible for recovery of such sites by microbial transformation of both organic compounds and heavy metals into non-toxic products. These strategies depend mainly upon the catabolic biological activities of the microorganisms, and therefore their ability to utilize the contaminants as nutrients and energy sources (Atlas and Unterman, 1999; Boopathy, 2000).

It is important to consider that when allochthonous microorganisms are incorporated into a soil, they usually cannot fully participate in the community activity in a meaningful way. This is why the use of indigenous microorganisms in bioremediation processes is so important. The actinobacteria are a group of bacteria that is found in high concentrations in soils. They play an important ecological role in recycling substances in the natural world, using humic acids for their growth as well as organic matter, which is difficult to degrade (Kieser et al., 2000). The physiological diversity of actinobacteria allows the production of a large number of metabolites with biotechnological importance included antibiotics, which are synthesized and excreted into a medium (Goodfellow et al., 1988; Ensign, 1990; Genilloud et al., 2011). The important role played by actinobacteria in the environment is also demonstrated by their ability to remove oil, rubber, plastics, pesticides, and heavy metals, among other substances (Goodfellow et al., 1988; Vobis, 1997; Benimeli et al., 2003, 2006, 2007a; Albarracín et al., 2005, 2010b; Polti et al., 2009, 2011).

There have been previous studies focused on biotransformation of OPs by actinobacteria, particularly in relation to lindane degradation (Benimeli et al., 2006, 2007a; Fuentes et al., 2011; Saez et al., 2012). *Streptomyces* spp. M7, A2, A5, and A11, isolated from sediments and soils contaminated with OPs, were found to be able to degrade lindane, as revealed by the release of chloride ions when the microorganisms were grown on media containing this pesticide as a sole carbon source (Benimeli et al., 2003, 2006; Cuozzo et al., 2009; Fuentes et al., 2010). Biotransformation of heavy metals [Cu (II), Cd (II), and Cr (VI)] by actinobacteria, particularly in terms of uptake and/or reduction to less toxic forms, has also been studied (Polti et al., 2007; Albarracín et al., 2008a; Siñeriz et al., 2009). *Streptomyces* sp. MC1, isolated from contaminated sugar cane, has shown the ability to reduce Cr(VI) to Cr(III) in both liquid and solid culture media (Polti et al., 2009, 2010). *Amycolatopsis tucumanensis* DSM 45259, isolated from sediments contaminated with heavy metals has also shown resistance to copper and chromium under a variety of culture conditions (Albarracín et al., 2005, 2008b, 2010a).

Table 1
Actinobacteria strains used in this study.

Strain	GenBank accession number	Reference
Streptomyces sp. M7	AY459531	Benimeli et al., 2003
Streptomyces sp. MC1	AY741287	Polti et al., 2007
Amycolatopsis tucumanensis DSM 45259	DQ886938	Albarracín et al., 2005
Streptomyces sp. A2	GU085103	Fuentes et al., 2010
Streptomyces sp. A5	GQ867050	Fuentes et al., 2010
Streptomyces sp. A11	GQ867055	Fuentes et al., 2010

The use of a single population involves many metabolic limitations, which could be avoided by using a mixed community of microorganisms. In nature, microorganisms exist as elements of microbial consortia, made up of multiple populations that coexist and carry out complex chemical processes and physiological functions in order to enable survival of the community. Microbial consortia can combine the catalytic specialties of different species to metabolize new substrates, including pesticides (Dejonghe et al., 2003; Smith et al., 2005; Yang et al., 2010; Fuentes et al., 2011; Shong et al., 2012). A microbial consortium formed by resistant actinobacteria could thus enhance the potential to simultaneously remove Cr(VI) and lindane. In the present work, authors therefore experiment with the bioremediation of soil contaminated with Cr(VI) and lindane using actinobacteria previously identified and characterized.

The effect of metals on biodegradation is still a matter requiring further study, in order to develop cost-effective remediation processes for sites co-contaminated with metals and organic pollutants. The impacts that metals have on biodegradation are complex and are influenced by the matrix structure, which determines the bioavailable metal concentrations. Metals inhibit biodegradation using different mechanisms and patterns, which depend upon the biological and physico-chemical characteristics of each system. A variety of approaches to bioremediation of co-contaminated sites are under development, and they include addition of metal-resistant microorganisms as well as additives that reduce metal bioavailability (Sandrin and Hoffman, 2007).

Several authors have evaluated bioremediation in media co-contaminated with metals and persistent organic compounds. Olaniran et al. (2009) investigated the impact of lead and mercury on biodegradation of 1,2-dichloroethane in soils, and they concluded that heavy metals have a negative impact on this bioprocess. These authors also found that biostimulation can have a positive influence on 1,2-dichloroethane degradation.

Another emerging approach is bioaugmentation. Sprocati et al. (2012) used this strategy to remediate soils co-contaminated with diesel oil and heavy metals. The bioaugmentation was performed by introducing a consortium composed of 12 allochthonous bacterial strains, previously isolated from a site with long-term pollution. This strategy showed high efficiency in the bioremediation process.

The aim of this work was to evaluate the use of actinobacteria, as pure or mixed culture, to remediate soil co-contaminated with lindane and Cr(VI).