



## Review

# Actinobacteria: Current research and perspectives for bioremediation of pesticides and heavy metals



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## HIGHLIGHTS

- Pesticides and heavy metals are prevalent contaminants found around the world.
- Bioremediation using actinobacteria is a better option for cleanup polluted sites.
- Actinobacteria have physiological and metabolic properties useful for bioremediation.
- Successful bioremediation techniques are developed using actinobacteria.
- Multi-omics analysis will enable to design new strategies using actinobacteria.

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## ABSTRACT

Actinobacteria exhibit cosmopolitan distribution since their members are widely distributed in aquatic and terrestrial ecosystems. In the environment they play relevant ecological roles including recycling of substances, degradation of complex polymers, and production of bioactive molecules. Biotechnological potential of actinobacteria in the environment was demonstrated by their ability to remove organic and inorganic pollutants. This ability is the reason why actinobacteria have received special attention as candidates for bioremediation, which has gained importance because of the widespread release of contaminants into the environment. Among organic contaminants, pesticides are widely used for pest control, although the negative impact of these chemicals in the environmental balance is increasingly becoming apparent. Similarly, the extensive application of heavy metals in industrial processes lead to highly contaminated areas worldwide. Several studies focused in the use of actinobacteria for cleaning up the environment were performed in the last 15 years. Strategies such as bioaugmentation, bio-stimulation, cell immobilization, production of biosurfactants, design of defined mixed cultures and the use of plant-microbe systems were developed to enhance the capabilities of actinobacteria in bioremediation. In this review, we compiled and discussed works focused in the study of different bioremediation strategies using actinobacteria and how they contributed to the improvement of the already existing strategies. In addition, we discuss the importance of omic studies to elucidate mechanisms and regulations that bacteria use to cope with pollutant toxicity, since they are still little known in actinobacteria. A brief account of sources and harmful effects of pesticides and heavy metals is also given.

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## 1. Introduction

In the last two decades ecofriendly methods have emerged for cleaning up contaminated environments using different microbial species. This approach, known as bioremediation, is generally considered to be less invasive and more restorative of soil functions compared to conventional physicochemical methods (Kidd et al., 2009). Bioremediation as sustainable technology becomes important analyzing the high release of anthropogenic chemicals into the environment.

Pesticides are chemicals used for pest control, and are probably the most widely distributed contaminants in the environment. The disposal of obsolete pesticide stocks has also resulted in many long-term contaminated sites with very high levels of these compounds. Officially recognized sites, typically dominated by organochlorine (OC) pesticides, have been reported in Brazil, Argentina, Chile (Barra et al., 2006), Poland (Gałuszka et al., 2011), Spain (Concha-Graña et al., 2006), The Netherlands (van Liere et al., 2003), China (Zhu et al., 2005), Canada, USA (Phillips et al., 2006), and India (Singh et al., 2007), among others. However, these reports underestimate the real situation because of the presence of illegal contaminated storage sites. For instance, the most important known illegal disposal of more than 30 tons of OC pesticides [lindane ( $\gamma$ -HCH), chlordane (CLD), methoxychlor (MTX), aldrin, DDT] and several heavy metals [Cr(VI), Cu(II), Cd(II)], has been found in the southeast of Santiago del Estero, Argentina (Chaile et al., 1999; Fuentes et al., 2010). Meanwhile, pollution arising from agricultural activities is considered diffuse since the compounds are distributed over large areas and at low concentrations. Residues of pesticides have been reported for many countries in air (Lammel et al., 2007), water (Kumari et al., 2007), soil (Fuentes et al., 2010), food commodities (Bajpai et al., 2007), milk (Zhao et al., 2007), fishes (Malik et al., 2007), and even in human blood and adipose tissue (Ridolfi et al., 2014).

Heavy metal pollution is also one of the most significant environmental problems today. Contaminated sites can be remediated by a wide range of technologies, however, in case of metal contamination, only a few technologies can be applied because of

the immutable and generally immobile character of metals (Dávila Costa et al., 2011a,b). The wide use of heavy metals in several applications leads to their worldwide distribution in soil, silt, waste, and wastewater. The pollution of the environment by toxic metals arises as a result of several human activities, largely industrial, although sources such as agriculture, municipal landfill, and sewage disposal also significantly contribute (Fernández et al., 2014).

Mixed pollution caused by the presence of organic and inorganic compounds tends to be concentrated in industrial zones, oil storage areas, waste recycling sites, and soils and sediments near roads (Mansour, 2012). For instance, several heavy metals and OC pesticides were found in water and silt samples from a major river basin from Northern Argentina ("The Salí basin"), at concentrations up to 10 times higher than allowed by law (Polti et al., 2007). Co-pollution is a very important problem because more than one third of polluted sites have more than one type of contaminant (Mansour, 2012).

Actinobacteria are a group of bacteria present in high concentrations in soils. They play an important role in recycling substances, since they are able to metabolize complex organic matter (Kieser et al., 2000). The important ecological role played by actinobacteria is demonstrated by their capability to remove xenobiotics compounds such as pesticides, and heavy metals, among others substances (Albarracín et al., 2005; Benimeli et al., 2003, 2006, 2007; Polti et al., 2009, 2011a,b, 2014). Because of their metabolic versatility, actinobacteria have received great global interest for several biotechnological applications.

This review focus on how different techniques/strategies using actinobacteria can contribute to improve the bioremediation of pesticides and/or heavy metals. We will present evidence about biotransformation of these pollutants by free or immobilized single or mixed cultures, providing information about the ecotoxicological risks of byproducts of the process. In this context, attention will be paid to omics as they provide invaluable tools for answering what mechanisms and genetic/physiological regulations are behind the degree of resistance to different pollutants. This review compiles and updates information available on the role of plant-associated

actinobacteria for improving phytoremediation efficiency and success. The use of surface-active microbial compounds is also described as a promising alternative to increase the bioavailability of environmental pollutants. A brief account about the sources and disposal of pesticides and heavy metals as well as its harmful effects on human health is also given.

## 2. Sources and distribution of pesticides and heavy metals in the environment

### 2.1. Pesticides

Agricultural production is one of the largest and most important economic activities in the world therefore, since the 1950s the use of chemicals that control crop pests has continuously grown.

Multiple residues of pesticides discharged by industries or as result of their extensive use in agriculture have been monitored. These residues contaminate river ecosystems including sediments and aquatic biota, causing harmful effects in humans through food and drinking water (Chopra et al., 2011). At present, several freshwater bodies are contaminated with pesticides. Only about 10% of the wastewater produced by human activities is treated; the rest is discharged into the water system. In addition, subsurface run-off from agricultural fields contains a variety of fertilizers and pesticides that generally flow into local rivers. In the last five years, a great number of researchers have reported pesticide residues in groundwater and drinking water around the world. Moreira et al. (2012) revealed the presence of atrazine, endosulfan, and methyl parathion, among others, in groundwater and rainwater samples collected in two municipalities of Mato Grosso state (Brazil), situated among the major soybean, corn, and cotton producers in the country.

The water scarcity and over application of pesticides has nowhere been more complex than in China. An example of this problem was reported by Li et al. (2015), who demonstrated that both shallow and deep aquifers in the Shanxi Province (China) were contaminated with OC pesticides such as hexachlorocyclohexane isomers (HCHs), o,p'-DDD, aldrin, and endosulfan-sulfate. Besides, several researchers have reported pesticides residues in ground water and drinking water in India. Yadav et al. (2015) did an extensive review of the presence of persistent organic pesticides in multi-component environmental samples, finding that different freshwater bodies were contaminated with DDT, endosulfan, HCHs, parathion-methyl, among others, many of them proposed as persistent organic pollutants (POPs) by the 12th Stockholm Convention.

Pesticides movement in soil compartments depends on their solubility in water, their adsorption by soil particles, and their persistence. The organic matter content is a factor which define the pesticide retention in soil and sediments, because it provide a number of binding sites for organic pollutants, especially hydrophobic compounds. For instance, the retention of HCH isomers in different soil types is primarily governed by organic matter. In general, the contaminant retention by soil components leads to a decrease in bioavailability and hinders degradation (Becerra-Castro et al., 2013).

Monitoring of pesticide residues in soils and sediments were reported by many researchers through the world. More relevant studies about the presence of pesticides in different countries, during the last decade, are summarized in [Supplementary Table 1](#).

Many pesticides can volatilize from the soil and foliage, and move far away from the application area. This explains traces of pesticides detected in pristine regions, suggesting that their occurrence is due to the atmospheric redistribution rather than a result of direct application (Shegunova et al., 2007). Despite the

importance of this world environmental concern, the information published on pesticide residues in air is limited. Yadav et al. (2015) had reviewed the information available on POPs residues found in the Indian air and all the studies found high levels of these compounds. Zhang et al. (2008) monitored POPs along the coastal region of India, and they found DDT and its metabolites (DDTs), HCHs, and CLD.

In Africa, atmospheric measurement of banned pesticides and other class of pesticides (current use pesticides, CUPs) is also very limited. However, recently, Arinaitwe et al. (2016) reported that the air of Lake Victoria basin, a system that has a high level of commercial and subsistence agriculture activity, was contaminated with CUPs and OC pesticides. Chlorpyrifos (CPF) was the predominant CUP in air samples during the evaluated period (2008–2010).

In 2008, Spain began monitoring POPs in air since air was recognized as the major route of long-range transport of pesticides through the world, by the Global Monitoring Plan of the Stockholm Convention. In this context, passive air samplers were placed in seven remote points and four urban Spanish locations to assess levels of DDTs, hexachlorobenzene (HCB), and HCHs. Results revealed that HCB was the major pollutant, followed in decreasing order by HCHs, and DDTs when urban and remote locations were evaluated together. Urban areas presented statistically significant higher levels for all families studied, except for HCB, compared to remote locations revealing anthropogenic activities as potential sources for HCHs and DDTs (Torre et al., 2016). In contrast, Estellano et al. (2015) did not find any difference between urban and rural concentrations of CUPs (CPF, malathion, diazinon, trifluralin, among others) in air samples taken of ten sites in the Tuscan region, Italy, with CPF being the one with the highest concentrations in air.

### 2.2. Heavy metals

Heavy metals are present in water, soil, sediments, air, and living organisms and they have both natural and anthropogenic origin. Anthropogenic sources generate a constant and permanent increasing pollution, while the natural source is usually a seasonal phenomenon, influenced by weather, and generally does not generate pollution (Armah et al., 2014; Bradl, 2005; Wuana and Okieimen, 2011).

There are three major causes of anthropogenic pollution: industries, agriculture, and urbanization. Among polluter industries, the most important are tanneries, textiles, metallurgical, galvanizing factories, distilleries, and factories producing pesticides, fertilizers, paints, varnishes, and pharmaceuticals (Chabukdhara and Nema, 2012; Srinivasa Gowd et al., 2010). Metallurgical industries produce direct contamination during extraction, processing, and use of metals; however, most industries produce pollution indirectly. For example, when using fossil fuels for boilers, they cause the release of metals found in these fuels (Bradl, 2005; Wuana and Okieimen, 2011).

Textile industries and tanneries generate highly polluted effluents that reach water courses and affect even very remote areas. For instance, heavy metal contamination was measured in a tannery effluent-affected lagoon and canal water in the southwestern Dhaka (Bangladesh). The mean concentrations of Cr(VI), As(V/III), Pb(II), and Cd(II) exceeded allowed limits recommended by the Government of Bangladesh, and it was demonstrated that the effluents discharged from auxiliary industries and tanneries were the main sources of pollution (Bhuiyan et al., 2010). In mining, for every gram of metal extracted large amounts of waste rock are produced, containing low concentrations of heavy metals. These metals are mobilized by chemical or biological leaching and pass to the ground and surrounding waters. Finally they are incorporated into the food chain (Li et al., 2015). The mining problem affects even areas that

currently are not exploited. For example, in southern Poland, the sediments of the Matylda catchment were polluted with concentrations of Pb(II), Zn(II), and Cd(II) up to 1000 times higher than geochemical background values, probably as a consequence of the first discharge of mine waters (Ciszewski et al., 2012).

The main energy resource in China is coal, and it comes from underground mining. The soil is generally filled with coal gangue and other solid wastes, with high content of toxic heavy metals. As a result of leaching, heavy metals migrate to the lower soil layer. Studies performed in soils from several active and former coal mines in Huainan (China) showed the presence of Cd(II), Hg(II), Pb(II), and Cu(II) at concentrations above the background values of Huainan soil (Yao et al., 2010).

In Europe this phenomenon has also been observed. One example was highlighted in a study around the Monica mine (Spain). This mine was exploited since the seventeenth century to the twentieth century, first to obtain silver and then arsenic. In the surrounding soil, concentrations of divalent ions of Cd, Cu, Pb, and Zn much higher than reference values were detected (García-Salgado et al., 2011).

Agricultural practices also contribute to heavy metal pollution through the use of pesticides, fertilizers, and soil amendments. Natural phosphate fertilizers can contain heavy metals as impurities. Nziguheba and Smolders (2008) evaluated the heavy metal content in approximately 200 P-fertilizers of western European countries and they detected Ni(II), Cd(II), Zn(II), Pb(II), As(V/III), and Cr(VI). Repeated use of these compounds generates toxic accumulation in soils (Wuana and Okieimen, 2011). Similarly to fertilizers, pesticides may also contain heavy metals as impurities. Furthermore, there are different inorganic pesticides that contain As(V/III), Hg(II), Pb(II), and Cu(II) as active ingredients. Due to high toxicity, the use of pesticides containing Hg(II) and Pb(II) is already banned, however, because of persistence of these metals, it is still possible to find them in agricultural soils and surrounding areas (Paranjape et al., 2014).

Urbanization generates large amounts of waste, wastewater, and sewage sludge. A common practice is the land irrigation with municipal and industrial wastewater. Although the metal concentrations in wastewater are generally below the toxic levels, long-term irrigation results in heavy metal accumulation in the soil (Kothe et al., 2010). On the other hand, inadequate disposal of municipal solid waste produces contaminated leachate. Fernández et al. (2014) have reported ground water and surface water pollution by leachate in a municipal landfill, a leachate column contained high concentration of Fe(III), Mn(II), and Cr(VI), that reached the main river course of drinking water in the area.

Electronic waste is an emergent problem. Electronic waste contains high concentrations of metals which are released when these materials are improperly treated. In the vicinity of an abandoned e-waste recycling site in China, surface soil was heavily contaminated with Cd(II) and Cu(II), which exceeded their respective guideline levels (Wu et al., 2015).

Natural sources of heavy metals include different types of rocks, volcanic eruption, mineral deposits erosion, oceanic evaporation, and general pedogenic processes (Bradl, 2005; Zeng et al., 2014). The natural concentrations of heavy metals vary considerably from one area to another, and the chemical composition of the parent rock determines the content of heavy metals in the soil, while the climate and soil organisms affect the speciation of metals. Baseline content of heavy metals in soil is almost always lower than the allowable levels (Bradl, 2005; Zeng et al., 2014). However, soils with high concentrations of several metals have been reported. Liaghati et al. (2004) evaluated the heavy metal content in a non-industrialized coastal plain from Queensland (Australia). They found elevated levels of Cr(VI), V(V), Ni(III), Cu(II), Zn(II), Pb(II),

As(V/III), Fe(III), and Al(III), which correlated with the composition of parent rock; thereby, it was concluded that those levels were probably of natural origin.

### 2.3. Mixed pollution

In general, there are few examples of point source pollution by only metals or only pesticides, mixed pollution is most frequently found. For instance, according to data from the Environmental Protection Agency, 40% of hazardous waste sites included in the National Priority List is contaminated with organic compounds and heavy metals (Olaniran et al., 2013). This situation generates a greater challenge at the time of recovering the health of the environment. Moreover, several authors have detected mixed pollution not only in industrial areas but also in remote locations due to the drift of these pollutants. Supplementary Table 2 summarizes relevant data on mixed water, sediment, and soil contamination reported in the last five years around the world.

## 3. Harmful effects of pesticides and heavy metals on human health

### 3.1. Pesticides

Ideally a pesticide must be lethal to the target pests, but not to non-target species, including humans. Unfortunately, this is not the case and inadequate management of pesticides constitutes potential occupational hazards and environmental risks for ecosystems (Lake et al., 2012). Besides, pesticide residues retained in crops can directly influence public health via food consumption. The World Health Organization has reported that occupational poisoning by pesticides has resulted in a million cases worldwide and provided evidence that pesticides were responsible for pathologies severely affecting many aspects of human health (WHO, 1990). In fact, pesticide pollution has been implicated in the rise of 'cancer villages'. This term refers to a place in which the mortality rate of cancer is significantly higher than the average, probably because a widespread pesticide contamination, mainly in water (Lu et al., 2015).

According to their toxicity, the most toxic pesticides are (in decreasing order) the organophosphorous (OP), carbamate (CB), and OC pesticides. These compounds are powerful chemicals that act primarily by disrupting nervous system function (Ridolfi et al., 2014). Organophosphorus and CB insecticides act as acetyl cholinesterase inhibitors, with a consequent elevated level of acetylcholine, affecting several organs such as peripheral and central nervous systems, muscles, liver, pancreas, and brain; whereas OC insecticides are neurotoxic involved in alteration of ion channels. As a shared mechanism, OP, CB, and OC insecticides induce cellular oxidative stress via affecting mitochondrial function and therefore disrupt neuronal and hormonal status of the body (Karami-Mohajeri and Abdollahi, 2010).

High doses of OP insecticides cause acute intoxication. The symptoms of this kind of poisoning include gastrointestinal upset, bronchospasm, miosis, urination, sweating, lacrimation, bradycardia, fasciculations, muscle weakness, hypertension, central nervous systems depression and coma. In contrast, people with chronic OP exposures develop a pesticide-related illness, which includes symptoms as nausea, headache, dizziness, blurred vision, abdominal pain, vomiting, and chest tightness (Sullivan and Bloese, 1992).

Exposure to CB insecticides is associated with the development of respiratory diseases. Nine of 12 studies investigating the effects of asthma found a positive association between CB insecticides and atopic asthma. This association was found for occupational,



domestic, and environmental exposures. Moreover, studies of occupationally exposed populations linked OP and CB insecticides in particular to obstruction and to decrease mid expiratory flow rates (Sanborn et al., 2012).

Organochlorine insecticides constitute a serious environmental problem and considerable risks for human health. This is because its biological degradation is difficult, they are highly soluble in lipids (and consequently biomagnify in the food chain), and they are persistent in the environment because of its chlorinated nature (Wang et al., 2013). Consequently, OC pesticides exert many toxic effects on human health (mainly as endocrine disruptors) producing infertility, cancer of reproductive systems, neurotoxicity, and immunotoxicity.

### 3.2. Heavy metals

Some heavy metals are essential elements for the existence of all known life forms since they perform several functions in biological systems. However, many others do not have a known biological function (Singh et al., 2011). Cells possess homeostatic mechanisms to regulate the concentrations of heavy metals and minimize the toxic effects produced by excessive levels. Heavy metal toxicity depends upon the absorbed dose, the route of exposure, and duration of exposure. The poisoning with some heavy metals can occur, for example, from drinking water. This is the case of lead poisoning in which acute exposure can cause loss of appetite, hypertension, renal dysfunction, fatigue, arthritis, hallucinations, and vertigo. Chronic exposure to lead can result in mental retardation, birth defects, psychosis, autism, allergies, weight loss, hyperactivity, paralysis, muscular weakness, and may even cause death (Martin and Griswold, 2009).

Mercury is considered one of the most toxic heavy metals, and has the ability to combine with other elements and form organic and inorganic mercury. Mercury is released into the environment by the activities of diverse industries, and in most marine species it is often seen at higher concentrations with increasing trophic levels (Hosseini et al., 2013). Organic mercury can permeate across the biological membranes due to its lipophilic nature, and at elevated levels it can damage the nervous system altering brain functions and lead to shyness, tremors, memory problems, irritability, etc. (Alina et al., 2012).

Other heavy metals have, at low concentrations, a defined biological role. Chromium, for instance, can occur in many different states, being Cr(VI) and Cr(III) the most stable forms in the nature (Colin et al., 2012). While Cr(III) is an essential nutritional supplement for animals and humans and has an important role in glucose metabolism, Cr(VI) is highly toxic and has been listed as a priority pollutant and human carcinogen by the US Environmental Protection Agency. When Cr(VI) contacts with broken skin, it can result in the formation of ulcers, which heal very slowly.

The implications of heavy metals in the development of other specific pathologies resulting on the oxidative stress induced by free radical formation have been also reported. Redox reactions arising from transition metals, such as Cu(II) and Zn(II), are the main chemical origin of radicals and reactive oxygen species (ROS). Heavy metals such as copper, lead, mercury, among others, have been linked to schizophrenia and atherosclerosis (Santos-Gallego and Ishwarlal Jialal, 2016). ROS generation can cause oxidation of low density lipoproteins and damages the vascular wall, resulting in plaque formation in the arteries.

In summary, health consequences resulting from the exposure to pollutants are mainly due to the lack of information, failure to apply the existing laws, inadequate supervision and lack of awareness of the problem. Thereby, more effective legislation, population monitoring programs and detection of the areas where

there are higher levels of contaminants, are undoubtedly necessary.

### 4. Bioremediation: a convenient solution to the problem of pesticides and heavy metal pollution

The scientific world can boast that science was behind many of the most important events in the history of humanity. The application of scientific principles to the processing of materials by biological agents to generate a better quality of life is generally known as “Biotechnology”. Empirically at the beginning, humans have applied biotechnology since our existence on Earth. Historically, biotechnology has been associated with food. Over the past years, biotechnology increased its fields of application and hence the relationship with society.

Environmental biotechnology, usually bioremediation, may be defined as the “use of living organisms to clean up pollutants from soil, water, or wastewater” (EPA, 2016). Overall, bioremediation may be considered as a “treatment that uses naturally occurring organisms to transform hazardous substances into less toxic substances”. Based on ancient reports, bioremediation was applied for the first time by the Roman people to treat their waste water. However, since 1972 bioremediation has been intensively applied as a means of cleaning up polluted systems.

Bioremediation of toxic organic compounds is often less controversial than bioremediation of heavy metals. This is because an organic compound can be completely degraded to carbon dioxide and water by a process known as mineralization. However in some cases, it may occur that microorganisms do not complete the mineralization and produce intermediaries more toxic than the original compound. This can be solved by further biotreatments. As for heavy metals, the situation is markedly different from the organic compounds since metals cannot be mineralized. Thus, the controversy arises; is it possible to bioremediate heavy metals? Are we really solving the problem? Bioremediation of metals such as Cr(VI), Cu(II), As(V), and Cd(II) is based on their conversion to a less toxic form and/or immobilization in order to reduce their bioavailability.

The world requires a higher production of food and an increase of the industrial activity in order to satisfy basic needs of the population. A consequence of these anthropogenic activities is that areas contaminated with toxic organic and inorganic compounds have been detected worldwide. This simultaneous contamination, known as co-contamination, represents the real current challenge of grey biotechnology. Bioremediation of organic compounds and heavy metals has been shown to be successful, although at the moment each process has generally been performed singly. Certainly, a multifunctional biological process is needed for bioremediation of co-contaminated sites. In this sense, bacteria of the phylum Actinobacteria show great promise because they have been demonstrated to be efficient tools for bioremediation of pesticides and heavy metals. Currently, members of the phylum Actinobacteria are being studied because of their ability to bioremediate co-polluted soils (Aparicio et al., 2015; Polti et al., 2014).

### 5. Actinobacteria: general features of the phylum

The phylum Actinobacteria constitutes one of the main and most diverse phyla within the domain Bacteria, on the basis of its branching position in 16S rRNA gene tree. The group encompasses six classes, 19 orders, 50 families, and 221 genera, although new taxa continue to be discovered (Goodfellow et al., 2012).

The actinobacteria are Gram-positive or Gram-variable aerobes, facultative anaerobes or anaerobes, which have a rigid cell wall that contains muramic acid. Most of them are chemo-organotrophs and free-living members of the phylum were universally recognized as

high G + C organisms, however, this paradigm was relatively recently broken since cosmopolitan and abundant fresh water actinobacteria have low content of G + C in their genomes (Ghai et al., 2012). The phylum includes phenotypically diverse organisms which exhibit a wide variety of morphologies that range from cocci to highly differentiated mycelia and spore production which could be advantageous for long-distance dispersal. The taxon exhibits a cosmopolitan distribution, with members of the group being widely distributed in aquatic and terrestrial ecosystems (Goodfellow et al., 2012). Specially in soil, they are important organisms mediating formation of soil organic matter. Different life styles are encountered among actinobacteria, saprophytic free-living aquatic and soil actinobacteria as well as plant commensals, nitrogen-fixing symbionts, gastrointestinal tract inhabitants, and animal and plant pathogens are counted among the group (Fiedler et al., 2005; Goodfellow et al., 2012). Nowadays, its members are considered among the most successful colonizers of all environments in the extremobiosphere, in opposite to the traditional perception of actinobacteria as autochthonous soil and freshwater organisms.

Actinobacteria exhibit diverse physiological and metabolic properties, such as the production of extracellular enzymes and the formation of a wide variety of secondary metabolites (Goodfellow et al., 2012). In fact, members of the order Actinomycetales, notably the *Streptomyces* genus, remain the richest source of natural products, including clinically useful antibiotics, antimetabolites, and antitumour agents (Bérdy, 2005; Olano et al., 2009). Actinomycetes produce about 45% of all microbial bioactive secondary metabolites, with 80% of these compounds being produced by the *Streptomyces* genus (Bérdy, 2005). Actinobacteria also produce non-antibiotic molecules that exhibit bioactivity, such as enzyme inhibitors, immunosuppressors, phytotoxins, biopesticides, biosurfactants, nano-particles, probiotics and enzymes involved in the degradation of complex polymers (Manivasagan et al., 2013). This versatility in secondary metabolite production makes them important tools for pharmaceutical, medical, and biotechnological applications such as bioremediation.

### 5.1. Promising genera for bioremediation of heavy metals

Heavy metal stress is a key environmental factor determining the structure and function of microbial communities (Hemme et al., 2010). Such structure can be evaluated through either culture-dependent or culture-independent methods, both presenting limitations. Culturable microorganisms represent only 0.1–1% of the total microbial population, while independent culture methods do not provide information about the physiological state of the non-culturable fraction (Handelsman, 2004). According to Ellis et al. (2003), plate counts seem to be a more appropriate technique for detecting the effect of metals on soil bacteria than culture-independent methods. They found that metal pollution did not have a significant impact on genetic diversity but affected physiological conditions. In this sense, Margesin et al. (2011) determined that the physiologically active fraction in sites contaminated with heavy metals was not only represented by Proteobacteria (as other research has indicated) but also by actinobacteria. Similar results were found by Oliveira and Pampulha (2006). The quantitative analysis of soil microbial populations through total culturable numbers showed a marked decrease of the different microbial groups for contaminated soil samples, in comparison with uncontaminated samples. However, actinobacteria showed less sensitivity than other culturable heterotrophic bacteria and asymbiotic nitrogen fixers. Culture dependent methods have allowed the isolation and characterization of over 35 genera of actinobacteria tolerant to heavy metals (Supplementary Table 3).

Although the Actinobacteria phylum comprises six classes, metal tolerant microorganisms have only been detected within the class Actinobacteria, except for two strains of the genus *Acidimicrobium*, belonging to the class Acidimicrobiia. *Acidimicrobium ferrooxidans* is an extremophile able to grow at pH 1.8 and 45 °C. The collection strain *Acidimicrobium ferrooxidans* DSM 10331T was tolerant up to Zn(II)  $33 \times 10^3 \text{ mg L}^{-1}$ , while *A. ferrooxidans* N39-30-03, isolated from a spent copper sulfide heap, was tolerant to higher concentrations of several metals, including Zn(II), demonstrating its strong adaptation to the adverse environment (Mangold et al., 2012).

Within the class Actinobacteria, members tolerant to heavy metals have been found in 10 of the 16 orders. Within the order Actinomycetales, is the genus *Actinomyces*, known for its human and animal pathogenicity. *Actinomyces turicensis* AL36Cd was isolated from soils contaminated with several heavy metals and showed tolerance to high concentrations of Cd(II) (Oyetibo et al., 2010).

*Propionibacterium freudenreichii shermanii* JS and *Bifidobacterium breve* Bbi99/E8 belong to the orders Propionibacteriales and Bifidobacteriales, respectively, and are used as probiotics (Halttunen et al., 2008). These strains have showed ability to remove Cd(II) and Pb(II) from aqueous solution. Because of its proven safety, the use of these strains in the removal of heavy metals has advantages over the use of *Actinomyces* strains.

*Frankia* species, belonging to the order Frankiales, are environmentally relevant bacteria whose potential could be exploited in revegetation of disturbed ecosystems (Bélanger et al., 2011). In this context, the tolerance of *Frankia* to several heavy metals such as Cd(II), Co(II), Cu(II), Cr(VI), Ni(II), and Zn(II) has been evaluated and demonstrated (Bélanger et al., 2011; Rehan et al., 2014; Wheeler et al., 2001). *Frankia* populations have shown ability to survive in highly polluted soils and to form effective symbioses if metal tolerant actinorhizal host plant species are planted for reclamation purposes (Wheeler et al., 2001).

Three genera belonging to Pseudonocardiaceae family (order Pseudonocordiales) have showed ability to grow in presence of heavy metals, namely *Amycolatopsis*, *Lentzea*, and *Saccharothrix* (Albarracín et al., 2005; Haferburg et al., 2009; Hamedí et al., 2015). Particularly, the strain *Amycolatopsis tucumamensis* DSM 45259<sup>T</sup> has demonstrated a high adaptation towards copper, which includes copper accumulation, up regulation of genes to antioxidant proteins like superoxide dismutase, alkyl hydroperoxide reductase and mycothiol reductase, and also, cupric reductase activity (Dávila Costa et al., 2011a; 2012). It is remarkable that this strain was isolated from a drainage channel that receives effluents from a copper filter plant (Albarracín et al., 2005). Meanwhile, four strains belonging to the genera *Lentzea* and *Saccharothrix* were isolated from different high metal contaminated soils from Argentina, Germany, and Iran. They have shown tolerance to B(III), Cd(II), Cu(II), Ni(II), or Zn(II), in assays carried out on solid culture medium (Haferburg et al., 2009; Hamedí et al., 2015; Moraga et al., 2014).

Within the order Corynebacteriaceae, seven genera tolerant to heavy metals have been described, belonging to five families, the most important of which are *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Rhodococcus*. There are several reports on *Corynebacterium* strains tolerant to heavy metals; for instance *Corynebacterium kutscheri* FL108Hg, isolated from the water and sediments of sewerage from allied-chemical industries (Nigeria), is resistant to Cd(II), Co(II), Cr(VI), Hg(II), and Ni(II) (Oyetibo et al., 2010). Several multiple metal resistant strains belonging to *Nocardia* and *Rhodococcus* were isolated from the same location. Other *Rhodococcus* metal resistant strains were also isolated worldwide from highly contaminated sites and from Antarctic sediments (Lo Giudice et al., 2012). Moreover, *Tsukamurella paurometabola* A155 is a rare

actinobacteria isolated from zinc mine in Thailand. This strain has shown ability to growth in presence of Cd(II) or Zn(II) and to bioaccumulate these metals (Limcharoensuk et al., 2015).

Micrococales is the order presenting the highest diversity within the phylum Actinobacteria. It has been found strains resistant to heavy metals in nine of the 15 families belonging to this order. Within the group, *Arthrobacter* is the second most important genus, after *Streptomyces*, in relation to tolerance to heavy metals and its potential use in bioremediation. The use of living cells, not viable biomass, cell-free extract, and even products as exopolysaccharides was assessed by several authors. The special features of *Arthrobacter* growth make it a model for genetic studies and for application in bioremediation processes. The environmental prevalence of *Arthrobacter* may be due to its ability to survive long periods under stressful conditions, such as starvation, desiccation, temperature shifts, oxygen radicals, and toxic chemicals, including heavy metals (Henne et al., 2009). The Cr(VI) resistance has been observed in several *Arthrobacter* strains, mostly isolated from chromate contaminated matrices. In this sense, Camargo et al. (2004) isolated *Arthrobacter crystallopoietes* ES 32 from a dichromate contaminated soil. This strain was able to grow in the presence of Cr(VI) and to reduce it to Cr(III), through a periplasmic chromate reductase stimulated by NADH. Because *Arthrobacter* tolerate alkaline conditions, which are common in soils contaminated with chromium, the authors propose the use of both, intact cells and cell-free extract for the bioremediation of alkaline soils contaminated with chromate. *Arthrobacter rhombi*-RE was also isolated from a chromium-contaminated site. This strain could reduce Cr(VI) through a reductase activity associated with the cell-free extract. The activity was enhanced by NADH and Ca(II), but inhibited by other divalent cations as Hg(II), Cd(II), Ba(II), and Zn(II). The cell-free extract containing the enzyme was successfully immobilized in different matrices, and was even used with continuous flow (Elangovan et al., 2010).

In view of the numbers of strains found with ability to reduce chromium, several studies have been conducted to determine the molecular origin of these characteristics. Patra et al. (2010) found a chromate reductase in *Arthrobacter aurescens* MM10 almost identical to another one from *E. coli*, although their activities were significantly different, indicating that the Cr(VI) reduction could be influenced by the presence of other genetic elements. A different Cr(VI) resistant mechanism has been described by Henne et al. (2009) for *Arthrobacter* sp. FB24. This strain extrudes chromate through a ChrA transport protein, regulated by genes involved on a signal transduction system.

A desirable feature in microorganisms feasible for bioremediation is the capability to be resistant to several metals. In this connection, *Arthrobacter ramosus* was isolated from mercuric salt-contaminated soil and could reduce and detoxify Cr(VI) and Hg(II); chromate reductase and mercuric reductase activities were found in the cell-free extract. MerA enzyme, responsible of Hg(II) reduction, was partially purified and its identity was confirmed being the first report on characterization of MerA enzyme from an *Arthrobacter* sp. (Bafana et al., 2010).

Because of their several biotechnological applications, the most important genus within the phylum Actinobacteria is *Streptomyces*, and this is no exception regarding the bioremediation of heavy metals (Table 1). The study of *Streptomyces* for bioremediation includes the use of metabolically active cells, their products, and dead biomass. High concentrations of metals may not be compatible with some living systems, hence using products or biomass as adsorbents can be a good alternative. In this sense, Chergui et al. (2007) reported the use of *S. rimosus* biomass obtained after antibiotics production to successfully remove several divalent metals, individually and mixed.

In contrast, to carry out bioremediation processes with metabolically active cells it is necessary first to determine the tolerance or resistance of the microorganisms to heavy metals and then, it is also necessary to assess whether the mechanism involved in this process is useful for the purposes of bioremediation. One of the first systematic studies on the tolerance to heavy metals in *Streptomyces* was carried out by Abbas and Edwards (1990). They evaluated the toxicity of Hg(II), Cd(II), Co(II), Zn(II), Ni(II), Cu(II), Cr(VI), and Mn(II), on 34 streptomycete species representative of different taxonomic cluster groups. Among the most important findings they reported that species that appeared to be the most tolerant or sensitive to one metal also exhibited a similar response to other metals. Thus, it is of interest to investigate about the tolerance of *Streptomyces* isolated from contaminated sites, since theoretically they should have adaptive mechanisms to grow under those adverse conditions. Several authors have followed this approach. Amoroso et al. (1998) have isolated 33 actinobacteria from sediments of Río Hondo reservoir (Tucumán, Argentina), a site contaminated with heavy metals and pesticides. They found six strains resistant to Cu(II), Cd(II), and Hg(II); they also determined that the mechanisms of Cu(II) and Cd(II) tolerance include biosorption processes and subsequent bioaccumulation. In another study, 25 *Streptomyces* strains were isolated from soil samples of the former uranium mine Wismut (Thuringia, Germany). The strains showed high resistance to Ni(II), probably due to the presence of high affinity Ni-transporter genes which provide a means to remove excess nickel ion from the cells (Amoroso et al., 2000). Among the different isolates were the strains *Streptomyces* sp. F4 and *S. acidiscabies* E13. The last one was the first strain reported with ability to produce a new biomineral containing nickel, named Ni-struvite. Moreover, the mineral formation was dependent on the biological activity and was postulated as a resistance mechanism, which immobilizes the metal and therefore decreases its toxicity. On the other hand, *Streptomyces* sp. F4 was able to uptake and complex Cd(II) from culture medium and soil samples (Haferburg et al., 2008). Similarly, Polti et al. (2007) have evaluated Cr(VI) resistance in *Streptomyces* strains and detected higher Cr(VI) specific removal by strains isolated from polluted areas compared to those isolated from non polluted sites, which could establish a relationship between the environmental conditions and adaptive responses. Moreover, *Streptomyces* sp. MC1, isolated from sugar cane plant grown on a polluted area, was able to reduce Cr(VI) in liquid minimal medium and soil samples (Polti et al., 2009, 2011b). This ability has been attributed to an intracellular enzyme or linked to the membrane, which use NADH as electron donor (Polti et al., 2010). Furthermore, *Streptomyces* sp. MC1 showed ability to immobilize chromium in soil and improve maize plant development (Polti et al., 2011b).

Although many *Streptomyces* with resistance to heavy metals have been isolated, the mechanisms involved are still little understood; therefore further studies are needed to improve its application in bioremediation. In this connection, Álvarez et al. (2013) built the largest *Streptomyces* phylogeny in combination with genomic, physiological, and biochemical data in order to unravel the mechanisms associated to the evolution of heavy metal resistance in the *Streptomyces* genus. Besides determining *Streptomyces* is a monophyletic group, the authors found that strains presenting resistance to heavy metals are widespread across *Streptomyces* phylogeny suggesting that the resistance to metals could have been originated many times by different mechanisms in *Streptomyces* evolution.

## 5.2. Promising genera for bioremediation of pesticides

Actinobacteria have received attention as candidates for bioremediation of different environmental compartments polluted with recalcitrant inorganic and organic compounds (Álvarez et al.,

**Table 1**  
*Streptomyces* strains useful in bioremediation of heavy metals and metalloids.

Metal	<i>Streptomyces</i> strain	Isolation sample	Mechanism	Reference	
As(V)	VITDDK3	Marine soil samples collected at the Ennore saltpan	ND	Lakshmiopathy and Kannabiran (2010)	
B(III)	<i>Streptomyces</i> sp. (8 strains)	B-contaminated soils, Salta, Argentina	ND	Moraga et al. (2014)	
Cd(II)	<i>S. viridochromogenes</i>	ND	Biosorption	Rho and Kim (2002)	
	<i>S. rimosus</i>	Biomass produced during oxytetracyclin antibiotic production collected after fermentation	Biosorption	Selatnia et al. (2004)	
	K33	Industrial metal mine	Biosorption	Yuan et al. (2009)	
	F4	Uranium mine	Biosorption/Bioaccumulation	Siñeriz et al. (2009)	
	VITDDK1	Marine soil samples collected at the Ennore saltpan	ND	Lakshmiopathy et al. (2010)	
	VITDDK2	Culture collection	Biosorption	Veneu et al. (2012)	
	<i>S. lunalinharesii</i>	Zinc–copper mine, Shaanxi province, Northwestern China	Biosorption/Bioaccumulation	Lin et al. (2012)	
	<i>S. zinciresistens</i>				
	CCNWNQ0016 <sup>T</sup>				
	Cr(III)	VITSVK9	Marine sediment, Bay of Bengal, India	Biosorption	Saurav and Kannabiran (2011)
	Cr(VI)	3M	Chromate contaminated soil	Reduction	Das and Chandra (1990)
		R22	Sediment samples from	Bioaccumulation	Amoroso et al. (2000)
R25		Salí River, Tucumán, Argentina			
<i>S. thermocarboxydus</i> NH50		Soil contaminated by leaking drums of metal finishing effluents, Lyon, France	Reduction by agents present in the supertant	Desjardin et al. (2003)	
C35		Sediment samples from the reservoir El Cadillal, Tucumán, Argentina	Efflux	Polti et al. (2007)	
M40		Copper filter plant, Tucuman, Argentina	Reduction	Polti et al. (2007)	
M46					
MC2		Sugar cane plant	Reduction	Polti et al. (2007)	
MC3					
MC1		Sugar cane plant	Reduction/bioaccumulation	Polti et al. (2007, 2010, 2011a,b)	
<i>S. rimosus</i>		ND	Biosorption	Chergui et al. (2007)	
MS2		Marine sediment	Reduction	Mabrouk (2008)	
<i>S. griseus</i> NCIM 2020		National Collection of Industrial Microorganisms, Pune, India	Reduction	Poopal and Laxman (2009)	
VITSVK9		Marine sediment, Bengal, India	Biosorption	Saurav and Kannabiran (2011)	
RSF17		Saline farmlands, Punjab, Pakistan	Reduction	Javaid and Sultan (2013)	
CRF14					
<i>S. matansis</i> BG5					
<i>S. vinaceus</i> CRF2					
<i>S. pulcher</i> CRF17					
<i>S. griseoincarnatus</i> SCF18					
<i>S. violaceoruber</i> LZ-26-1	Lanzhou reaches of the Yellow River, Gansu, China	Reduction	Chen et al. (2014)		
<i>S. werraensis</i> LD22	Chicken and goat feces	Biosorption	Latha et al. (2015)		
Cu(II)	<i>S. viridochromogenes</i>	ND	Biosorption	Rho and Kim (2002)	
<i>S. coelicolor</i> A3(2)	Collection strain	Biosorption	Öztürk et al. (2004)		
<i>Streptomyces</i> sp.(9 strains)	Copper filter plant, Tucuman, Argentina	ND	Albarracín et al. (2005)		
<i>S. rimosus</i>		Biosorption	Chergui et al. (2007)		
AB2A	Copper filter plant, Tucuman, Argentina	Reduction	Albarracín et al. (2005)		
AB3					
AB5A					
A160	Bay of Bengal, India	ND	Yadav et al. (2009)		
A161	Pondicherry, India				
A164					
<i>S. flavovirens</i> ON3	Soil exposed to heavy traffic emissions, Brno, Czech Republic	Biosorption	Majzlik et al. (2011)		
<i>S. flavovirens</i> M4					
<i>S. zinciresistens</i> , CCNWNQ0016 <sup>T</sup>	Zinc–copper amine, Shaanxi, China	Biosorption/Bioaccumulation	Lin et al. (2012)		
<i>S. lunalinharesii</i>	Culture collection	Biosorption	Veneu et al. (2013)		
<i>S. acrimycini</i> NGP	Marine sediments,	Biosorption	Selvam and Vishnupriya (2013)		
<i>S. albogriseolus</i> NGP	Tamilnadu, India				
<i>S. variabilis</i> NGP					
Hg(II)	<i>S. coelicolor</i> M130	Culture collection	Enzymatic reduction	Nakahara et al. (1985)	
	<i>S. lividans</i> 1326				
	<i>S. lividans</i> strain 8				
	<i>S. espinosus</i> strain 5				
CHR 3	Site heavily contaminated with metals, Baltimore Inner Harbor, USA	ND	Ravel et al. (1998)		
CHR 28					
Ni(II)	<i>S. coelicolor</i> A3(2)	Culture collection	Biosorption	Öztürk et al. (2004)	
	<i>S. rimosus</i>	Biomass produced during oxytetracyclin production and collected after fermentation	Biosorption	Selatnia et al. (2004)	
	<i>S. aureofaciens</i> NR-3	Riparian sediments contaminated with high levels of Ni and U, Steed Pond, USA	Ni-influx and Ni-efflux transporters would be present to maintain homeostasis	Van Nostrand et al. (2007)	
	<i>S. galbus</i> NR-2		Biominer production	Haferburg et al. (2008)	
	<i>S. acidiscabies</i> E13	A former uranium mining area, Thuringia, Germany			



Table 1 (continued)

Metal	<i>Streptomyces</i> strain	Isolation sample	Mechanism	Reference
Pb(II)	<i>S. viridochromogenes</i>	ND	Biosorption	Rho and Kim (2002)
	<i>S. rimosus</i>	Biomass produced during oxytetracyclin production and collected after fermentation	Biosorption	Selatnia et al. (2004)
	<i>Streptomyces plumbiresistens</i> CCNWHX13-160	Lead-polluted soil, Gansu, China	ND	Guo et al. (2009)
Zn(II)	<i>S. rimosus</i>	Biomass produced by an antibiotic production and collected after fermentation	Biosorption	Mameri et al. (1999)
	<i>S. viridochromogenes</i>	ND	Biosorption	Rho and Kim (2002)
	<i>S. rimosus</i>	Biomass produced during oxytetracyclin production and collected after fermentation	Biosorption	Chergui et al. (2007)
	<i>S. flavovirens</i> ON3	Soil exposed to heavy traffic emissions, Brno, Czech Republic	Biosorption	Majzlik et al. (2011)
	<i>S. flavovirens</i> M4			
	<i>S. zinciresistens</i> CCNWNQ0016 <sup>T</sup>	Zinc–copper mine, Shaanxi, China	Biosorption/ Bioaccumulation	Lin et al. (2012)
	<i>S. lunalinharesii</i>	Culture collection	Biosorption	Veneu et al. (2013)
	<i>S. acrimycini</i> NGP	Marine sediments, Tamilnadu, India	Biosorption	Selvam and Vishnupriya (2013)
	<i>S. albogriseolus</i> NGP			
	<i>S. variabilis</i> NGP			

2012b). In this connection, De Schrijver and De Mot (1999) provided a comprehensive review on pesticide degradation by actinobacteria. However, several advances in the use of actinobacteria in pesticides decontamination have occurred in the subsequent 15 years, and diverse techniques have been incorporated for the enhancement of the capabilities of actinobacteria in bioremediation strategies.

Analyzing the bibliography published in the last two decades, it can be noted that the genera *Arthrobacter*, *Rhodococcus*, *Streptomyces*, *Frankia*, *Janibacter*, *Kokuria*, *Mycobacterium*, *Nocardia*, and *Pseudonocardia*, are the most representative pesticide-degrading actinobacteria. These microorganisms exhibit a wide range of activities and have the ability to grow and degrade several chemical families of pesticides, including OC, CB, OP, pyrethroids, ureas, and chloroacetanilides. Table 2 shows the main genera of pesticide-degrading actinobacteria, isolation sites, culture conditions, examples of pesticides degradation, and major observations.

The *Arthrobacter* genus has been recognized as degrader of different xenobiotics, since members of this group possess various catabolic pathways for the detoxification of these compounds, being most of them plasmid-encoded. Microorganisms of this genus are ubiquitous due to their nutritional versatility and tolerance to environmental stress. Thus, Sagarkar et al. (2016) reported the isolation and characterization of *Arthrobacter* sp. AK-YN10 with the ability to degrade atrazine in 24 h, transforming it to cyanuric acid. In addition, AK-YN10 has shown to degrade several other s-triazines including simazine, ametryn, prometryn, ametryn, prometryn, and terbuthylazine. Southern blot analysis confirmed the localization of the *trzN*–*atzBC* degrader gene combination into a single plasmid. Also, De Paolis et al. (2013) reported two *Arthrobacter* species (*A. fluorescens* and *A. giacomelloi*) that were able to metabolize HCH isomers, being *A. giacomelloi* the most effective one, since it reached 88% degradation of  $\alpha$ , 60% of  $\beta$ , and 56% of  $\gamma$ -HCH after 72 h of incubation. The formation of possible persistent compounds was also studied and pentachlorocyclohexenes and tetrachlorocyclohexenes have been detected as metabolites, being almost completely eliminated after 72 h of incubation, while phenolic compounds were not found.

Another OC pesticide that could be degraded by an *Arthrobacter* strain is endosulfan. Kumar et al. (2008) isolated an *Arthrobacter* strain that showed 73% of biodegradation of  $\alpha$ -endosulfan and 75% of  $\beta$ -endosulfan, after six weeks of incubation. *Arthrobacter* sp. oxidized endosulfan to the toxic compound endosulfan sulfate, which was further metabolized.

Urea pesticides, like diuron, are relatively persistent in soil, and

the major route for its natural attenuation in the environment is microbial degradation. Devers-Lamrani et al. (2014) characterized the diuron-degrading potential of *Arthrobacter* sp. BS1, BS2, and SED1 by sequencing the *puhA* gene. The authors found that this gene had 99% similarity to *A. globiformis* strain D47 *puhA* gene, previously isolated in the UK (Cullington and Walker, 1999), suggesting that diuron-degrading ability could be specific to *Arthrobacter* genus.

Among actinobacteria, rhodococci have useful industrial and/or ecological applications due to their variety of metabolic activities. Members of the genus *Rhodococcus* are ubiquitous in fertile soil and may be also present in polluted environments where they play an important role in the degradation of different pollutants. Also, rhodococci are promising candidates for bioremediation because these bacteria can resist starvation conditions in soil while the presence of more easily assimilable carbon sources may not adversely affect the breakdown of pollutants. The hydrophobic nature of *Rhodococcus* cells is due to the presence of aliphatic chains of mycolic acids in the cell wall which may be helpful in the degradation of less soluble pesticides. Kolekar et al. (2013) studied the biodegradation of atrazine by *Rhodococcus* sp. BCH2, demonstrating that the actinobacterium was capable of utilizing the pesticide as a carbon and nitrogen source. Toxicity tests using *Eisenia foetida* with a seven-day-old culture of biodegradation assay showed less toxic response as compared to those treated with atrazine without microorganism, which suggests that *Rhodococcus* sp. BCH2 could be efficient for degrading atrazine also *in vivo*. Besides, Verma et al. (2006) isolated *Rhodococcus* MTCC 6716 strain from the gut microflora of an Indian earthworm (*Metaphire posthuma*). Endosulfan was used by this microorganism as carbon source and it was degraded up to 93% within 15 d. Furthermore, the persistent form of the toxic metabolite endosulfan sulfate was not produced. Degradation of the pesticide occurred simultaneously with an increase in chloride ion (87%) in the growth medium, suggesting nearly complete degradation of the insecticide. Later, Verma et al. (2011) observed that endosulfan degradation was mediated through genes present in genomic DNA and the expression of marker gene was found to be endosulfan concentration dependent.

Besides *Arthrobacter* and *Rhodococcus* genera, microorganisms of the genus *Streptomyces* have received considerable attention as an effective biotechnological approach to clean up polluted environments. In addition to their metabolic diversity, strains of *Streptomyces* may be well suited for soil inoculation as a consequence of their mycelial growth habit, relatively rapid growth rates,

**Table 2**  
General characteristics of main genera of pesticides-degrading actinobacteria.

Microorganism	Pesticide	Isolation sample	Culture conditions	Reference
<i>Arthrobacter</i> sp. strain AK-YN10	S-triazine (atrazine)	Agricultural field repeatedly treated with atrazine in sugarcane cultivation, India	BS medium, atrazine (1000 mg L <sup>-1</sup> ), 30 °C Soil microcosms, atrazine (100 mg kg <sup>-1</sup> ), 30 °C, 40% humidity	Sagarkar et al. (2016)
<i>Arthrobacter fluorescens</i> (DSM 3680) and <i>Arthrobacter giacomelloi</i> (DSM 3681)	Organochlorines ( $\alpha$ , $\beta$ , $\gamma$ -hexachlorocyclohexane)	Pristine soil	Mineral salt medium, hexachlorocyclohexane isomers (100 mg L <sup>-1</sup> ), 28 °C	De Paolis et al. (2013)
<i>Arthrobacter</i> sp.	Organochlorines ( $\alpha$ , $\beta$ -endosulfan)	Soil from different agricultural fields contaminated with pesticides, India	Soil microcosms (sandy loam soil), endosulfan (50 mg kg <sup>-1</sup> ), 37 °C, 30% humidity	Kumar et al. (2008)
<i>Arthrobacter</i> sp. BS1, BS2 and SED1	Urea (diuron)	Soil from the interface between a vineyard and the Morcille River, France	Mineral salt medium, diuron (25 mg L <sup>-1</sup> ), 28 °C	Devers-Lamrani et al. (2014)
<i>Rhodococcus</i> sp. BCH2	S-triazine (atrazine)	Long-term atrazine-treated grape farm soil, India	Atrazine synthetic medium, atrazine (100 mg L <sup>-1</sup> ), pH 7, 30 °C, darkness	Kolekar et al. (2013)
<i>Rhodococcus</i> sp. MB-P1	S-triazine (atrazine)	Contaminated soil from Markfed Agrochemicals, India	Minimal salt medium, atrazine (200–1000 mg L <sup>-1</sup> ), 30 °C	Fazlurrahman et al. (2009)
<i>Rhodococcus</i> MTCC 6716	Organochlorine (technical endosulfan)	Gut microflora of an earthworm ( <i>Metaphire posthuma</i> ), India	Mineral medium, diuron (80 $\mu$ g mL <sup>-1</sup> ), 28 °C	Verma et al. (2006, 2011)
<i>Streptomyces aureus</i> HP-S-01	Pyrethroid (deltamethrin)	Activated sludge samples from an aerobic pyrethroid-manufacturing wastewater treatment system in Zhongshan, China	Mineral salt medium, deltamethrin (50 mg L <sup>-1</sup> ), 18–38 °C, pH 5–10, and inocula biomass 0.1–1.0 g dry wt L <sup>-1</sup>	Chen et al. (2011)
<i>Streptomyces</i> sp. M7	Organochlorine (lindane)	Wastewater sediment from a copper filter plant, Argentina	Minimal medium, lindane (100 $\mu$ g L <sup>-1</sup> ), 30 °C Soil microcosms, lindane (100, 150, 200, and 300 $\mu$ g kg <sup>-1</sup> ), 28 °C, 20% humidity	Benimeli et al. (2006) Benimeli et al. (2008)
<i>Streptomyces</i> sp. A2, A5, and A11	Organochlorine (lindane)	Pesticides contaminated soil, Argentina	Minimal medium, lindane (1.66 mg L <sup>-1</sup> ), 30 °C	Fuentes et al. (2011)
<i>Streptomyces</i> sp. AC1-6 and ISP4	Organophosphorus (diazinon)	Soil exposed to continuous applications of chlorpyrifos, Chile	Minimal medium, diazinon (25 and 50 mg L <sup>-1</sup> ), 28 °C	Briceño et al. (2013)
<i>Janibacter</i> sp. AS23	Pentachlorophenol	Sediments from arid and saline ecosystems, Tunisia	Mineral salt medium, pentachlorophenol (50, 100, 200, and 300 mg L <sup>-1</sup> )	Khessairi et al. (2014)
<i>Frankia alni</i> ACN14a and <i>Frankia</i> sp. Eul1c	S-triazine (atrazine)	Not reported	Buffer with atrazine (216, 647 and 1078 mg L <sup>-1</sup> ), 33 °C with and without C and N sources	Rehan et al. (2014)
<i>Gordonia</i> sp. JAAS1	Organophosphorus (chlorpyrifos)	Soil from a paddy field exposed to continuous applications of chlorpyrifos, India	Mineral salt medium, chlorpyrifos (110 mg L <sup>-1</sup> ) 28 °C	Abraham et al. (2013)

colonization of semi-selective substrates, and their ability to be genetically manipulated (Shelton et al., 1996). Different *Streptomyces* strains have the ability to grow on and degrade several classes of pesticides including OC, OP, CB, pyrethroids, atrazine, diuron, among others. For instance, in a study performed by Chen et al. (2011), a strain identified as *Streptomyces aureus* HP-S-01 was able to degrade deltamethrin and its major hydrolysis product 3-phenoxybenzaldehyde was oxidized to form 2-hydroxy-4-methoxy benzophenone, resulting in its detoxification. Strain HP-S-01 was also found highly efficient in degrading other pyrethroids like cyfluthrin, bifenthrin, cypermethrin, among others.

Biodegradation of OC pesticides by *Streptomyces* was also extensively studied. In this context, Benimeli et al. (2003) isolated 93 strains belonging to the Actinobacteria phylum. These bacteria were tested against 11 OC pesticides such as aldrin, CLD, dieldrin, DDTs, heptachlor and its epoxides, MTX, and  $\gamma$ -HCH. Four of these strains, identified as *Streptomyces* sp., were selected based on its multi-pesticide-tolerance. Among these, *Streptomyces* sp. M7 had the ability to use  $\gamma$ -HCH as the only carbon source in minimal medium.  $\gamma$ -HCH removal induction was observed, showing the maximum Cl<sup>-</sup> release when the pesticide was added to the medium at 20 h of incubation rather than 6 h (Benimeli et al., 2006). This strain was also evaluated in soil microcosms assays, showing  $\gamma$ -HCH removal percentages ranging from 14 to 78%, depending on the concentration of pesticide used (Benimeli et al., 2008). Subsequently, Cuzzo et al. (2009) demonstrated the presence of a dechlorinase enzyme in *Streptomyces* sp. M7 which was induced when the microorganism was grown with  $\gamma$ -HCH as the only carbon source. Fuentes et al. (2010) also isolated pesticide-degrading actinobacteria from soil samples collected from an illegal

pesticides storage in Santiago del Estero, Argentina. Most of the isolates belonged to the *Streptomyces* genus, and they were all able to grow in the presence of CLD,  $\gamma$ -HCH, and MTX, and to remove them from liquid medium. Nine of these strains could biodegrade one or more OCs, which was evidenced by the release of Cl<sup>-</sup> ions.

Briceño et al. (2013) studied OP pesticides biodegradation by two strains, identified as *Streptomyces* sp. AC1-6 and ISP4. They were able to remove approximately 70–90% of the initial diazinon concentration after 96 h of incubation. The pesticide removal was accompanied by microbial growth of the strains and rapid glucose consumption in the liquid medium during the first 24 h of incubation, reaching the depletion of almost all the glucose at the end of the experiment. The process of diazinon degradation was linked with the decrease of pH values, after glucose utilization, as was demonstrated for other microorganisms such as *Serratia* and *Pseudomonas* (Cycoñ et al., 2009).

Halophilic and halotolerant microorganisms play important roles in the transformation and degradation of organic pollutants in saline and arid environments. For instance, several species of the *Janibacter* genus were reported for their ability to degrade a large spectrum of aromatic and/or chlorinated compounds including polychlorinated biphenyls (PCB), monochlorinated dibenzo-p-dioxin, dibenzofuran, dibenzo-p-dioxin, carbazole, diphenyl ether, fluorine, and PAHs. Khessairi et al. (2014) isolated the pentachlorophenol (PCP)-degrading actinobacterium *Janibacter* sp. AS23. The strain was halotolerant and able to degrade up to 300 mg L<sup>-1</sup> of PCP, while the addition of Tween 80 enhanced the low soluble PCP removal capacity.

Microorganisms of the genus *Frankia* are well known for their symbiotic association with more than 220 species of woody plants

and their ability to fix atmospheric N<sub>2</sub> in the free-living state (*ex planta*) or in symbiosis (*in planta*) (Rehan et al., 2014). However, despite the importance of these bacteria in soil, there has not been much progress in the study of their ability to biodegrade pesticides. Rehan et al. (2014) demonstrated that the strains *Frankia alni* ACN14a and *Frankia* sp. Eu1c have both the ability to degrade atrazine via dechlorination and dealkylation. Analysis of the *Frankia* genome revealed for the first time the involvement of the enzyme adenosine aminohydrolase 3 in the dealkylation and deamination of s-triazine compounds.

Species of the genus *Gordonia* are also known as degraders of recalcitrant pollutants. These bacteria can grow in unfavourable environments, making them capable of living in a wide range of ecological niches. Abraham et al. (2013) isolated the strain designed as *Gordonia* sp. JAAS1, which was able to degrade the OP insecticide CPF within 24 h of incubation, with the accumulation of its main metabolite 3,5,6-trichloro-2-pyridinol (TCP) in the culture medium. This byproduct is more mobile than CPF due to its higher water solubility thus, causing widespread contamination. However, after 72 h of incubation, TCP was completely degraded by *Gordonia* sp. JAAS1.

These are just some examples demonstrating the great ability of actinobacteria to remove both pesticides and heavy metals, and thus its usefulness for the bioremediation of these pollutants. Although the great advances carried out in the last two decades, most of the mentioned works are limited to laboratory scale. Deeper studies must be done on a field scale based on the excellent results obtained in laboratory.

## 6. Strategies of bioremediation using actinobacteria

### 6.1. Division of labor: the use of defined mixed cultures

In natural environments, communities dominate the microbial world. Interactions among bacterial communities include touching through dedicated signals, horizontal gene transfer, competitive or cooperative scenarios where microbes compete for or provide resources, and alteration of environment to influence the growth of neighbors (Hays et al., 2015). Thus, efforts have been recently focused in employing microbial consortia for environmental remediation and wastewater treatment since they can perform complicated functions which are difficult or even impossible for individual strains (Brenner et al., 2008). Microbial communities possess an increase of metabolic capabilities allowing members of communities divide labors and survive perturbations (Hays et al., 2015). Division of labor is crucial especially in bioremediation; however the behaviour and role of the members of bacterial communities are completely enigmatic. Construction of a defined mixed culture would facilitate the examination of the characteristics of each one of its members, and the monitoring of their dynamics together, after evaluating the compatibility among the strains (Fig. 1). Furthermore, by constructing a “knockout community”, in which one of the members is eliminated from the defined mixed culture, it could be possible to evaluate the role played by the eliminated member and its impact on the others (Kato et al., 2005).

It has been demonstrated that actinobacteria are excellent candidates for bioremediation. As for pesticide bioremediation, some studies showed the advantages of using actinobacteria consortia. Thus, biodegradation of OC pesticides, such as lindane, MTX and CLD, by pure cultures and defined consortia of *Streptomyces* was studied (Fuentes et al., 2011, 2013a, 2016). First, the authors evaluated the compatibility among the strains to further assays as mixed culture (Fig. 1). In these works, pesticide removal and/or the specific dechlorinase activity (SDA) improved when actinobacteria were inoculated as consortia. For instance, when the defined mixed

culture composed by three actinobacteria were grown with chlordane, SDA increased more than 50-fold when compared with the pure cultures (Fuentes et al., 2016). Similar results were obtained in the degradation of  $\gamma$ -HCH by defined actinobacteria consortia (Fuentes et al., 2011). In turn, mixed cultures formed by two, three, and four strains were able to remove between 46 and 68% of  $\gamma$ -HCH, while pure cultures removed 37% as maximum. Similarly, a quadruple microbial consortium formed by *Streptomyces* strains efficiently removed MTX (Fuentes et al., 2013a). Certainly, actinobacteria working together enhanced pesticide removal and/or biodegradation.

In spite of pesticide removal studies being performed first individually, reality is quite different since environment is often contaminated with more than one pesticide. Application of actinobacteria for simultaneous removal of pesticide was also studied. Six actinobacteria were able to grow and/or remove a mixture of PCP and CPF using these pesticides as carbon source (Fuentes et al., 2013b). Furthermore, it was observed that three of the studied strains were unable to individually remove PCP, however by forming different consortia they removed 10.6% of this toxic and recalcitrant compound from the mixture. Devers-Lamrani et al. (2014) obtained a similar result in a study performed with a co-culture of the actinobacterium *Arthrobacter* BS2 and *Achromobacter* SP1. While neither of the strains could mineralize diuron, but equimolarly transformed it to its highly toxic main metabolite 3,4-dichloroaniline (3,4-DCA), together they entirely mineralized it, indicating a metabolic cooperation between these two populations.

Similar to pesticides, bioremediation of heavy metals seems to be more efficient when mixed cultures are used instead of pure cultures. As we mentioned previously, heavy metals can be removed by the cells through different mechanisms. These processes are favored by an increase in the number of cells, even more as consequence of a mixed culture. Recent works have demonstrated the success on the removal of heavy metals and metalloids by bacterial mixed cultures (Kiliç et al., 2015; Zhang et al., 2015). In spite of the excellence of these works and many others, results were obtained almost empirically since mechanisms or regulations were never proposed. Probably, the most important is to solve the

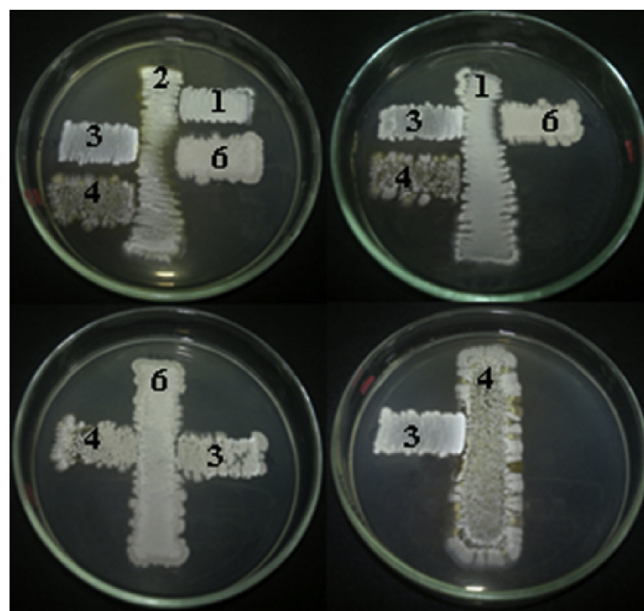


Fig. 1. Compatibility test among *Streptomyces* strains (1, 2, 4, 5, 6) and *Amycolatopsis tucumanensis* DSM 45259 (3). Strain N° 6 should not be included in the consortium since it inhibits the growth of the strains N° 1 and 2.



problem no matter how bacteria do it, however from a scientific point of view, the knowledge of mechanisms involved in any biological process results essential to make them more efficient. Individual mechanisms of resistance to heavy metals are well known and they include extrusion systems, reductases or oxidases, production of exopolysaccharide and synthesis of metallothioneins among others. Certainly, these mechanisms working together would lead to an extremely resistant bacterium; nevertheless cells do not often count with complete machinery. This is the point in which mixed cultures gain relevance since bacteria with incomplete mechanisms of resistance can complement each other and enhance their resistance. Interactive mechanisms linked to heavy metal resistance may vary or be regulated by several factors including concentration of the metals, nutritional state of the bacteria and physico-chemical conditions in general. However, some basic interactions may consist of: a) conversion of the metal to a less toxic form, extrusion and further immobilization in the exopolysaccharide produced by another bacterium; b) extracellular reduction or oxidation of the metal, absorption and intracellular immobilization by metallothioneins of second bacterium; c) biomineralization of the metal, in this case the rest of the bacteria could support chemical or nutritionally to the strain able to mineralize the metal. Overall, probability of occurrence of these processes should increase in bacterial mixed cultures of different genera since it is closer to reality. Thus, actinobacteria would be excellent candidates for these mixed cultures because of their proven versatility and abundance in the environment.

In this section we gave evidence of the advantages of using mixed cultures of actinobacteria in bioremediation. These benefits could be attributed to the positive interactions among members of the association (Abdualdaim et al., 2008). It is important to highlight that the community life could generate both robustness to environmental fluctuations and promote stability through time for the members of a consortium. Moreover, compared with monocultures, consortia might be more capable of resisting invasion by other species (Burmølle et al., 2006). All these emergent properties of microorganisms growing as a consortium are especially interesting for bioremediation processes. Based on the above, it is clear the complex nature of the environmental bioremediation and the advantages of using microbial consortia robust, stable, and with synergistic activity to remove toxic compounds.

## 6.2. Cell immobilization

Cell immobilization has been defined as the physical confinement of viable microbial cells to a certain defined space as to limit their free migration, while retaining the catalytic activities and enhancing both biological and physical stabilities of the cells (Martins et al., 2013). This promising technique offers several advantages compared with conventional suspension system, such as the retention of higher concentrations of microorganisms in the reactor, easier solid-liquid separation, high metabolic activity and higher cell viability (weeks or months) (Ahmad and Kunhi, 2011). Also, the immobilization matrix can alleviate physicochemical challenges, such as temperature, pH, and toxic substances, being the latter particularly interesting for its use in bioremediation processes. Moreover, immobilized microorganisms could be cost effective since they can be easily reused several times without significant loss of activity (Martins et al., 2013).

All these features have resulted in this technique receiving an increasing interest in different fields, including the treatment of wastewater, and polluted soils and sediments. Although the application of immobilized cells to environmental area is still in its preliminary stages, the results seen so far are promising (Martins et al., 2013). In this context, several researches revealed that the

immobilization of microbial cells for bioremediation purposes resulted in high performances, greater degradative enzymes production, increased tolerance to high concentrations of toxic compounds, absence of cell washout, and extended biochemical or biotransformation reaction time (Poopal and Laxman, 2009). Furthermore, the use of immobilized microbial cells provides, in general, high degradation efficiency and good operational stability (Ahmad and Kunhi, 2011).

The support selection is a crucial factor for cells immobilization process. For the treatment of polluted sites, support materials need to present several properties, such as being non-biodegradable, non-toxic, and non-polluting, as well as presenting high mechanical and chemical stability, high diffusivity, minimum attachment with other organisms, and preferably low cost price (Martins et al., 2013) (Fig. 2). In this sense, Vancov et al. (2005) postulated that beads are ideally suited for application and distribution in contaminated environments, mainly because they act as a slow-release inoculant for bacterial cell dispersal without significantly impacting on the environment. Particularly, encapsulation of actinobacteria has been successfully used as a slow release delivery system for the mineralization of a range of pollutants such as pesticides, among others. For instance, Vancov et al. (2005) showed that *Rhodococcus erythropolis* NI86/21 encapsulated on alginate beads has the potential to reduce atrazine levels in soil and liquid medium. Besides, they revealed that modifying the beads formulation by adding bentonite and skimmed milk resulted in hastened cell release and prolonged cell survival, respectively.

Bazot et al. (2007) evaluated the mineralization of diuron by a bacterial co-culture of the actinobacterium *Arthrobacter* sp. N4 and *Delftia acidovorans* W34, which individually degraded diuron to 3,4-DCA and mineralized 3,4-DCA, respectively. When both bacteria were immobilized together, the degradation rate of diuron was almost three times higher than that achieved by free cells. This was probably due to the creation of micro-environments within the beads, which allowed optimum expression of both bacteria due to a better level of oxygen and substrates. In a subsequent study, the authors postulated that the immobilization technique influenced diuron dissipation by modifying the global equilibrium between N4 and W34 along the incubation time (Bazot and Lebeau, 2009). Similarly, Saez et al. (2012) studied the ability of pure and mixed cultures of *Streptomyces* immobilized in different matrices to remove  $\gamma$ -HCH, and proved that the removal efficiency achieved by the microorganisms immobilized in all the supports tested was significantly higher than by free cells; although cells immobilized in cloth sachets showed the best behaviour. Besides, a *Streptomyces* consortium immobilized in this support could be reused in two additional cycles obtaining a maximum pesticide removal efficiency of 71.5%. This result seems very interesting emphasizing that one important advantage of cell immobilization consists on the extended or repeated use of the cells. Afterwards, the immobilized *Streptomyces* consortium carried out a successful remediation process in liquid and soil slurry reactors polluted with 50 mg kg<sup>-1</sup> of  $\gamma$ -HCH (Saez et al., 2014, 2015). In turn, the ability of the *Streptomyces* defined consortium to degrade lindane was demonstrated by the identification of three byproducts of  $\gamma$ -HCH degradation in the liquid medium, namely 1,2-dichlorobenzene, 1,4-dichlorobenzene, and  $\gamma$ -pentachlorocyclohexene, and by the determination of chloride ions released in the slurry system, thus demonstrating that  $\gamma$ -HCH was not only removed but also degraded by the consortium. Finally, a phytotoxicity assay with lettuce plants confirmed that the toxicity of both systems significantly decreased after the treatment with the immobilized *Streptomyces* consortium, i.e. the byproducts of lindane degradation identified in the bioremediated systems would be not toxic or less toxic than the parental compound (Saez et al., 2015).



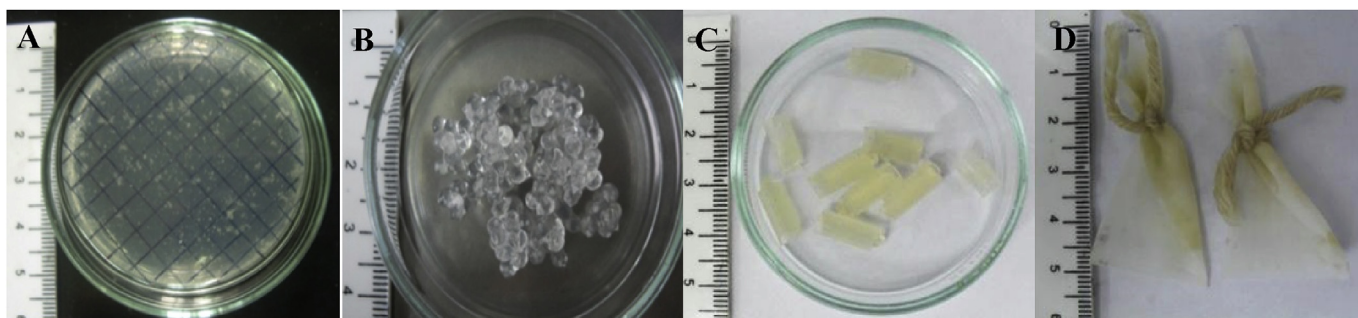


Fig. 2. Examples of immobilization supports. (A) Agar, (B) PVA-alginate, (C) Silicone tubes, (D) Cloth bags.

Furthermore, pure and mixed cultures of *Streptomyces* were also tested for the removal of other pesticides and even mixtures. For instance, Briceño et al. (2013) revealed that *Streptomyces* sp. AC1-6 immobilized in alginate beads exhibited almost 20% higher removal of diazinon from liquid medium than free cells. They also confirmed the reusability of the encapsulated cells obtaining more than 50% of pesticide removal after the third batch. Further, the removal of a mixture of CPF and PCP was assessed by using a mixed culture of *Streptomyces* strains immobilized in alginate beads. The removal efficiency increased more than 30% for CPF and almost 10% for PCP when using immobilized cells rather than free cells (Fuentes et al., 2013b).

In addition to pesticide removal, immobilization of actinobacteria also achieved good performances for metal bioremediation, providing higher metal resistance and enhanced metal accumulating ability (Ivshina et al., 2012). In this context, a chromate-reducing bacterium, *Microbacterium liquefaciens* MP30, was successfully entrapped in polyvinyl alcohol (PVA)-alginate beads, which was the most suitable support for cell immobilization and chromate reduction. Besides, chromate removal from a  $2.6 \text{ mg L}^{-1}$  solution was maintained at 90–95% over 20 days without signs of bead breakdown; although the immobilization technique had little effect on biological activity (Pattanapitpaisal et al., 2001). In contrast, Humphries et al. (2005) found that agar and agarose were the best immobilization matrices for *Microbacterium* sp. NCIMB 13776 for Cr(VI) reduction. Therefore, it could be concluded that the microorganism used may have a greater effect on the metal removal than the immobilization technique. In this sense, Jézéquel and Lebeau (2008) also found that the microorganism played the major effect upon the reduction of the phytoavailable Cd instead of the culture technique.

Poopal and Laxman (2008), meanwhile, also revealed that PVA-alginate was the most effective matrix, among others such as agar, agarose, and polyacrylamide-alginate, for the immobilization of a *Streptomyces griseus* strain, regarding beads integrity and chromate reduction. Furthermore, chromate reduction rates achieved by immobilized *S. griseus* were comparable to those obtained by free cells, suggesting that there was no diffusion constraint for access of chromate or glucose, which has been reported as a limitation of the immobilization technology. Besides, the immobilized cells could be used up to five reduction cycles without compromising on its performance, highlighting this technique as a promising system for development of a continuous bioprocess for the treatment of contaminated effluents. Subsequently, they demonstrated that both the biomass concentration and the metal concentration affected the chromate reduction efficiency by the immobilized cells, which increased with an increase in biomass concentration but decreased as Cr(VI) concentration increased (Poopal and Laxman, 2009).

All these reports confirm that the use of immobilized actinobacteria is a promising alternative for the bioremediation of

polluted sites, being efficiently reused for the removal of metals as well as pesticides. However, extensive studies are needed on the selection of the correct support depending on the pollutants present, the longevity and reusability of the immobilized cells, among other factors affecting the efficiency of the process.

### 6.3. Use of plant-microbe partnerships

In the last few years there has been a growing interest in the influence of microorganisms on plant growth and contaminant bioavailability and degradation. Consequently, more and more studies are focusing on the role of plant-associated microorganisms in improving phytoremediation efficiency (Kidd et al., 2009; Weyens et al., 2009). Phytoremediation techniques, based on the interactions between plants and microorganisms, have been proposed as cost-effective and ecofriendly methods for cleaning up polluted soils (San Miguel et al., 2013).

Microbe amended phytoremediation appears to be specially effective for organic contaminants, including POPs (Gerhardt et al., 2009). Several studies have demonstrated enhanced dissipation of POPs at the root-soil interface (Becerra-Castro et al., 2013; Gerhardt et al., 2009; Kidd et al., 2009). The rhizosphere effect is generally attributed to an increase in microbial activity caused by the release of plant root exudates (REs) containing enzymes, amino acids, carbohydrates, low-molecular-mass carboxylic acids, and phenolic compounds (Curl and Truelove, 1986). Regarding this approach, Álvarez et al. (2015a) found that four *Streptomyces* strains were able to grow in a hydroponic system with maize plants as the sole carbon source, confirming that the plants, and/or the REs released by them, represent a viable carbon and energy source for these microorganisms. In fact, in a previous experiment, Alvarez et al. (2012a) had assayed the effect of isolated maize REs on growth and  $\gamma$ -HCH removal by three *Streptomyces* strains. The authors found that *Streptomyces* sp. A5 showed maximum biomass and the highest pesticide dissipation (55%) in presence of REs. As part of this study, carbohydrates, proteins, and phenolic compounds were detected in the collected REs. Álvarez et al. (2015a) also cultivated a mixed culture of four *Streptomyces* strains with maize plants on soils artificially polluted with  $\gamma$ -HCH. Similar levels of  $\gamma$ -HCH dissipation were registered in both inoculated and non-inoculated planted systems, suggesting that pesticide removal was not significantly affected by the bacterial inoculant. However, the inoculation of the actinobacteria consortium led to an increase in the vigour index of the maize plants and protected them against the existing toxicity. In a phytoremediation context, consortia can provide multiple benefits to plants, including the synthesis of protective compounds, chelators for delivering key plant nutrients, and degradation of contaminants before they can negatively impact the plants (Gerhardt et al., 2009). Similar observations were made by Becerra-Castro et al. (2013), who inoculated substrates seeded

with *Cytisus striatus*, which growing spontaneously on HCH-polluted sites, with the endophytic actinobacteria *Rhodococcus erythropolis* ET54b and *Sphingomonas* sp. D4. The authors found that systems planted showed a higher removal of the HCH isomers (including  $\gamma$ -HCH) and that the bacteria protected the plants against the negative effects of the pollutant.

Several authors have demonstrated that endophytic bacteria contribute for biodegradation of toxic compounds and the plant-endophyte association can be exploited for the remediation of polluted systems (Weyens et al., 2009). In a recent work, Mesquini et al. (2015) isolated from sugarcane leaves, the endophytic actinobacterium *Streptomyces* sp. atz2, which was able to reduce in 98% the concentration of atrazine in culture medium under aerobic and microaerophilic conditions. Degradation of atrazine was also demonstrated when *Arthrobacter* sp. DNS10 was inoculated in contaminated soil microcosms, together with the plant *Pennisetum*. The authors have shown the efficiency of the plant-*Arthrobacter* joint interactions (98% of atrazine degradation) compared with single strain and single-plant effects (Zhang et al., 2014).

Plant-microbe interactions in relation to trace elements have been mostly approached within the context of phytoremediation. It has been suggested that soil microorganisms and, in particular, the active rizosphere bacteria might improve metal mobilization and uptake by plants (Gremion et al., 2003). This is the case of several actinobacteria, since members of this phylum are heterogeneously distributed in the rizosphere where secondary metabolite producers are enhanced (Basil et al., 2004). This feature is generally attributed to the production of plant growth promoting factors, like indoleacetic acid, or the production of metal binding or chelating compounds such as siderophores (Dimkpa et al., 2009).

Bacterial diversity from heavy metal-contaminated rhizosphere of the metal-hyperaccumulating plant *Thlaspi caerulescens* were analyzed and compared with that of contaminated bulk soil (Gremion et al., 2003). The most remarkable result was that sequences belonging to actinobacteria dominated both bulk and rhizosphere soil, indicating that actinobacteria might be a dominant part of the active bacteria in heavy metal-polluted bulk and rhizosphere soil. Similarly, Idris et al. (2004) identified a great number of different Ni-resistant bacteria in the rhizosphere of the Ni-hyperaccumulator *Thlaspi goesingense*. Cultivation of bacteria on Ni-containing medium resulted mostly in the isolation of the actinobacteria *Rhodococcus* spp. and *Okibacterium* spp. (Idris et al., 2004). Also, different communities were identified in the rhizosphere of other Ni-hyperaccumulators, like *Alyssum serpyllifolium*. Becerra-Castro et al. (2011) isolated and characterized Ni-resistant rhizosphere bacteria from two subspecies of *Alyssum serpyllifolium*. The most Ni-resistant bacteria were mainly strains of the *Streptomyces* and *Arthrobacter* genera.

On the other hand, Kuffner et al. (2008) characterized Zn-resistant endophytic and rhizospheric bacteria of Zn-accumulating willows. A great number of bradyrhizobia, beta-proteobacteria, actinobacteria and, strains of the Bacteroidetes/Chlorobi phyla were found in the rhizosphere, while endophytes included several actinobacteria. Mengoni et al. (2001) sampled rhizosphere and bulk soil in serpentine sites and Ni-resistant strains were isolated. In the rhizosphere, the cultivable Ni-resistant population was mainly composed of *Pseudomonas* strains, whereas in soil mostly *Streptomyces* strains were found. In general, bacteria obtained in this study were co-resistant to Cr and Co, although other tolerance combinations were also found, indicating independent evolution of heavy metal resistance. As was described, the development of phytoremediation systems where microorganisms -mainly actinobacteria-interact with plants is being increasingly considered as an option for dealing with the inherent weaknesses related to application of isolated elements.

#### 6.4. Application of surface-active compounds of microbial origin

The bioremediation concept includes not only the use of biological agents such as plants and microorganisms to remove and/or neutralize contaminants, but also the use of products derived from them. The use of microbial products instead of the whole cells for environmental remediation could have unquestionable advantages, since it is not required that the producer microorganisms have the ability to grow and survive in the contaminated sites (Colin et al., 2013a). Among microbial products with potential application in bioremediation technologies, a wide range of surface-active compounds (SACs) can be found. These are amphiphilic molecules that can be classified into two classes: low-molecular weight SACs, called biosurfactants (lipopeptide, glycolipids, phospholipids), which reduce surface tension at the air-water interface; and high molecular weight SACs known as bioemulsifiers (polysaccharides, lipopolysaccharides, proteins, lipoproteins, complex mixtures of these compounds), which are most effective at reducing the interfacial tension between immiscible liquids or at the solid-liquid interface. Biosurfactants often exhibit emulsifying capacity but bioemulsifiers do not always reduce the surface tension (Batista et al., 2010).

Surface-active compounds of biological origin are under increasing demand as natural alternatives to their synthetically produced counterparts because they exhibit lower levels of toxicity and higher biodegradability (Shafiei et al., 2014). In addition, they have been particularly characterized by their high stability in extreme conditions of pH, temperature, and salinity, properties that increase their application scope in diverse biotechnological areas (Colin et al., 2013b; Khopade et al., 2012). In the field of bioremediation, *in situ* microbial production of SACs can be promoted in order to increase the bioavailability of organic and inorganic pollutants (Gnanamani et al., 2010; Wattanaphon et al., 2008). However, a more pragmatic approach consist in the production of these biomolecules by microbial culture for their subsequent isolation and application in soil washing technologies, in absence of producer microorganisms in the washing process.

Surface-active molecules are able to form complexes with pollutants attached to soil matrix, and to promote their desorption towards the aqueous phase. Once in aqueous phase, the hydrophobic pollutants, e.g. pesticides, are stabilized inside the micelles of biosurfactants, which favours their solubility and subsequent removal during the washing process. Concerning the inorganic pollutants, such as heavy metals, they can be removed from soil by forming micelles with SACs. However, in the last case, polar groups of SACs are able to bind, mobilize, and stabilize the metals in the surface of micelles.

To date, many actinobacteria able to produce SACs have been isolated and identified as belonging to diverse genera. Among them, the marine actinobacterium *Nocardioopsis* sp. B4, isolated from the west coast of India, was cultivated on diverse carbon and nitrogen sources, detecting the maximum production with olive oil and ammonium chloride at a C/N ratio of 2:1 (Khopade et al., 2012). Also, *Brachybacterium paraconglomeratum* MSA21, isolated from a marine sponge, was recognized for its ability to produce biosurfactants by using industrial and agro-industrial wastes under solid state culture (Kiran et al., 2014). This finding could be crucial in order to encourage the inexpensive production of microbial SACs at a large-scale. Thereby, the selection of microorganisms capable of growing and producing surfactant/emulsifier molecules from low cost raw materials is one of the most attractive strategies to promote a sustainable production (Colin et al., 2013a, 2014).

Strains of other actinobacteria genera are also known as SAC producers. For instance, *Amycolatopsis tucumanensis* DSM45259 was demonstrated as bioemulsifier-producing microorganism

(Colin et al., 2013a). Moreover, the authors noted that when the strain was cultivated in a minimal medium but replacing glucose and L-asparagine by glycerol and urea, a significant increase in the bioemulsifiers production was detected. Assays of bioemulsifiers stability against extreme environmental conditions of pH, temperature, and salt concentration were also performed. In general lines, it was observed that biomolecules produced from glucose were slightly more stable than those synthesized from glycerol. Finally, the performance of *A. tucumanensis* DSM45259 bioemulsifiers for the recovery of copper and chromium from contaminated soils by using washing technology at a laboratory scale was analyzed. The authors found that the biomolecules were able to mediate Cr(VI) recuperation, with twice the removal percentage compared to that seen when using deionized water. However, they were not effective in removing Cu(II) from soil. In this connection, González Huecas et al. (2003) found that metals as Cr(III/VI), Cu(II), and Pb(II) are strongly retained to the soil, probably by specific adsorption. In contrast, the hexavalent chromium present in the soil has more mobility, which can be increased by the presence of the bioemulsifiers.

The *Streptomyces* genus has also demonstrated a great ability to produce bioactive metabolites, including SACs. For example *Streptomyces* sp. VITDDK3, isolated from the coastal region of Tamil Nadu, India, was screened and recognized as a potential biosurfactant producer by conventional screening methods (hemolytic activity, drop collapsing, and lipase production) as well as for its heavy metal resistance activity (Deepika Lakshmi et al., 2010). Bioemulsifier production by *Streptomyces* sp. SS 20, isolated from hydrocarbon contaminated soil, was also reported (Hayder et al., 2014). These authors detected a maximum emulsifier activity in a culture medium when sucrose and yeast extract were used as carbon and nitrogen sources, respectively; added with of kerosene as source of hydrocarbons. More recently, a potential biosurfactant producer named *Streptomyces* sp. MAB36 was isolated from marine sediment samples (Manivasagan et al., 2014). The culture conditions to enhance this production were optimized, and fructose and yeast extract were the best substrates for the biosynthesis of a glycolipid with surfactant activity. The production of SACs by *Streptomyces* sp. MC1 was also evaluated. It was initially assayed in a synthetic medium, demonstrating that factors such as pH, phosphate concentration, and the addition of calcium ion to the culture medium have a significant influence on emulsifier biosynthesis (Colin et al., 2013b). Subsequently, in order to increase the economic viability of the production processes, alternative substrates were assayed. Thus, the authors noted that when raw vinasse was added to the synthetic medium as a carbon source replacing the glucose, a significant emulsifying ability was detected in the supernatants (Colin et al., 2016). Therefore, the use of an effluent such as vinasse to formulate the culture media could represent an effective means of ensuring the sustainability of the production process for bioemulsifiers, while the effluent volume is also significantly reduced.

Regarding the role of microbial SACs on pesticides depuration, Álvarez et al. (2015b) reported the biodegradation of HCHs present in slurries by using *Sphingomonas* sp. D4 alone or in co-culture with two biosurfactant-producing actinobacteria, *Streptomyces* sp. M7 or *Rhodococcus* sp. ET54b. In general lines, the authors detected an improvement in the biodegradation of HCHs when *Sphingomonas* sp. D4 was co-inoculated with the biosurfactant-producing actinobacteria. This suggests an increase in the HCHs bioavailability, probably mediated by the action of SACs. Similarly, Fuentes et al. (2016) evaluated the emulsifying activity produced by a mixed culture of *Streptomyces* strains using glucose or CLD as carbon source. Although the authors did not find significant difference in the production related to the carbon source, the emulsion stability

formed with kerosene was substrate-dependent: only emulsifier molecules biosynthesized from chlordane were stable. Different properties of the bioemulsifiers, including the stability of their emulsions, suggest that their chemical nature could be modified by varying the composition of the production media. In fact, Colin et al. (2013a) emphasized in the production of chemically different molecules, according to the carbon substrate used.

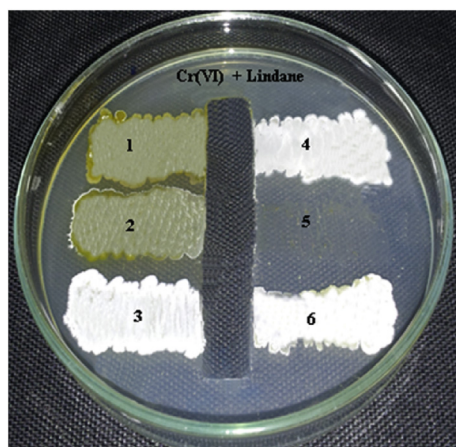
Despite the background on this topic, advances on pesticide remediation assisted by SACs produced by actinobacteria are still limited. In this sense, deeper studies are needed in order to detect new microbial SACs for the development of safer and cleaner technologies.

#### 6.5. Simultaneous bioremediation of pesticides and heavy metals

The restoration of environments co-contaminated with organic and inorganic pollutants through non-biological systems is a complicated endeavor because the methodologies used differ according to the physical and chemical characteristics of each kind of compound (Dong et al., 2013). In contrast, biological approaches are more flexible because living systems can perform complex reactions that include organic pollutants degradation and conversion of inorganic compounds into non-toxic products, simultaneously or sequentially (Chen et al., 2015a; Liu et al., 2015). As a first approach to achieve this purpose, the microbial tolerance to a mixture of pollutants must be assessed (Fig. 3). In this sense, several authors have studied complex mixtures bioremediation, using bacteria, fungi and/or plants (Chen et al., 2015b; Polti et al., 2014; Zhu et al., 2012).

The first barrier to overcome is the toxicity of metals on microorganisms. The presence of heavy metals could inhibit biodegradation of organic pollutants by either inhibiting enzymes involved in general metabolism or those directly involved in biodegradation (Olaniran et al., 2009). Metals inhibit microbial activity using different mechanisms, which depend upon the biological characteristics of each system. The impacts of metals on biodegradation are directly related to the bioavailable metal concentration, which varies with speciation, soil type, pH, redox potential, among others. Therefore, there are two main approaches to remediate co-contaminated environments: a) decrease metal bioavailability by addition of chelating or heat treatment, and b) biostimulation or bioaugmentation with metal resistant microflora. In this context, bioaugmentation with actinobacteria resistant to heavy metals, with ability to degrade pesticides is a promising approach. Nevertheless, there are very few attempts using actinobacteria for this purpose. For instance, Polti et al. (2014) evaluated different strategies to bioremediate soil co-contaminated with lindane and Cr(VI), by using six actinobacteria strains previously isolated from contaminated areas and selected based on their ability to degrade lindane (*Streptomyces* sp. A2, A5, A11, and M7), or remove metals (*Streptomyces* sp. MC1 and *Amycolatopsis tucumanensis* DSM 45259). The authors assessed the toxicity of both pollutants together, and observed that all strains, except for *Streptomyces* sp. A2, were resistant to both pollutants simultaneously, confirming the hypothesis that metal could be more toxic for this microorganism and hence inhibit its growth. The authors assayed single and mixed cultures of these bacteria in soil samples contaminated with Cr(VI) and lindane, using concentrations 5.5 and 2.5 times upper the permissible levels, respectively. In sterilized soil samples the ability of *A. tucumanensis* to remove Cr(VI) was inhibited in presence of lindane. This observation opposes to the previously described by other authors, where the metal inhibits microbial activity on the organic compound. Otherwise, it was expected that the consortium could remove more pollutants than individual strains; however this was only observed for chromium,





**Fig. 3.** Qualitative tolerance test of *Streptomyces* strains (1, 2, 4, 5, 6) and *Amycolatopsis tucumanensis* DSM 45259 (3) to Cr(VI) 500 mg L<sup>-1</sup> + lindane 250 µg L<sup>-1</sup>. Adapted from Polti et al. (2014).

while *Streptomyces* sp. M7 showed the best performance for lindane removal. In contrast, in non-sterilized soil lindane removal was higher for the consortium, while *Streptomyces* sp. M7 was more efficient in removing chromium. The authors hypothesize that the sterilization process not only eliminates the native soil flora but also changes its structure, and therefore the ability to immobilize the chromium affecting its bioavailability.

In order to understand the behaviour previously described it is necessary to take into account the interactions occurring in complex media such as soil. In this context, Aparicio et al. (2015) evaluated the influence of physical and chemical parameters, such as humidity, temperature, initial Cr(VI) and lindane concentration, on the bioremediation of lindane and Cr(VI) by *Streptomyces* sp. M7. They demonstrated that at high concentrations of both pollutants, the major Cr(VI) removal mechanism was physicochemical including high adsorption to soil particles and consequently low mobility; whereas, at low contaminant concentrations, Cr(VI) removal could respond to microbial activity. Moreover, they observed that lindane removal was increased at low humidity level, which is explained by its hydrophobic nature. The resistance of *Streptomyces* sp. M7 to Cr(VI) could protect its metabolic activity, and therefore, the ability to remove lindane. Finally, the authors assessed the success of the bioremediation by using *Lactuca sativa*. In complex systems, this represents a major challenge since the response is difficult to predict with high number of variables and interactions occurring, so, bioindicators represent an invaluable tool. Interestingly, at all tested conditions, the parameters of the plants grown on the bioremediated soil improved significantly, approaching to those grown on the uncontaminated control. According to these results, it could be inferred that there was an effective reduction of lindane and Cr(VI) bioavailability by *Streptomyces* sp. M7 in co-contaminated soil samples, with the consecutive generation of less toxic or non-toxic metabolites.

So far, these are the only reports on bioremediation of environments contaminated with pesticides and heavy metals by actinobacteria. The study of complex systems such as contaminated soil has two distinct but equally important approaches. One of them is the study of interactions between different biological and physicochemical factors for explaining the system performance and the metabolism of the bacteria involved in the bioremediation. The second aspect is the success of the bioremediation at different environmental conditions. Understanding the first will allow a major advance in the second, however, most of the studies on interactions are made in defined culture media, so that the results

cannot be extrapolated to soil. Therefore, bioremediation of soils is made with empirical considerations; thereby to further progress it needs the help of new methodologies such as metagenomics and metabolomics, among others.

## 7. Multi-omics analysis in bioremediation

### 7.1. Why omics?

In the last years, the field of biology has generated enormous amount of data based on genome-scale studies. Ever since, the suffix “-omics” has been used for studies undertaken on a genome-wide scale. Segen’s Medical Dictionary defines “omics” as a neologism for the constellation of an organism’s information, which includes the genome itself (genomic), transcription products (transcriptomic), protein products (proteomic), and metabolic products (metabolomic). According to the Oxford Dictionary, “genome” is the complete set of genes in a cell or living thing and it was a complete mystery until sequencing techniques started in the 70’s (Sanger and Coulson, 1975). In 1995, with the first bacterial genome sequencing using Sanger’s automated technologies sequencing (nowadays called first-generation sequencing), microbial genomics officially born (Fleischmann et al., 1995). Ever since, the number of sequenced bacterial genomes has increased exponentially every year and currently 12.3% of the bacterial genome projects belong to Actinobacteria (<http://www.genomesonline.org>). The valuable information generated by genome sequencing can be interpreted at transcriptional level by quantification of the expressed genes. Thus, transcriptome is a set of mRNA molecules that represents the genes actively expressed in a specific cellular stage. In this sense, microarrays of RNA were developed to interpret the function and patterns of regulation of genomes. However, limitations of microarrays such as the need to know the genome sequence and possible mistakes caused by crossing over led to directly sequencing transcripts of RNA (Wang et al., 2009).

Undoubtedly, the simultaneous sequencing of genomes and transcripts of RNA provide valuable insights into the physiological state of an organism. Nevertheless, high levels of mRNA transcripts do not ensure a high abundance of the respective protein or elevated activity in the case of enzymes. At this point, proteomics arose as a powerful tool. The term “proteome” was introduced in 1995 to represent the total of proteins synthesized by a cell in a specific moment upon a particular condition. Probably, proteomics is the richest omic concerning the variety of methodologies that have been developed. The conventional method to obtain a proteome was the two dimensional difference gel electrophoresis (2D-DIGE) for the separation of proteins and further identification (Unlu et al., 1997). Since the discovery of this technique in the 70’s, 2D-DIGE was widely used in the study of proteins, although it is time-consuming and sometimes difficult to reproduce. Nowadays, techniques that do not require a separation in gel (gel-free) have been already developed. Basically these methodologies include a trypsin digestion of the total of proteins and further identification by liquid chromatography coupled with mass spectrometry. Mass spectrometry (MS) based proteomics enables the identification of proteins regulated in response to physiological variation and/or stress conditions by an increase or decrease in their abundances. Advances in liquid chromatography, MS, and the available evaluation software make label-free proteomic feasible approaches. In addition to the detection of unmodified proteins by label-free proteomic, study of oxidative modification of proteins acquired relevance over the last few years. Oxidative modification often causes irreparable damage and can lead to inactivation, aggregation, and degradation of proteins. However, not all protein oxidations are irreversible. Particularly, oxidation of the thiol-group of



the amino acid cysteine can be reversed by dedicated antioxidant systems *in vivo*, such as the thioredoxin or glutaredoxin systems. In recent time it has become apparent that oxidative thiol modification plays an important role in redox regulation and provides a very effective and elegant mechanism for activity modulation of regulatory proteins. Thus, several methods including OxICAT were developed and successfully applied in the identification of redox-regulated proteins (Brandes et al., 2011; Dávila Costa et al., 2011a).

Metabolomics is probably the most recent omic and it was developed in order to elucidate the cellular metabolome. The nature and abundance of each metabolite present in a biological system represent the metabolome. Metabolome can also provide a detailed picture of the physiologic cellular state. Metabolic pathways include several metabolites such as carbohydrates, organic acids, amino acids, coenzymes, and lipids. For instance, 2708 y 2027 metabolites were identified in *E. coli* and *S. cerevisiae*, respectively (Guo et al., 2013; Jewison et al., 2012). Because of the dissimilar chemical nature of metabolites, simultaneous detection of a complex metabolome using a single technique results almost impossible. Thus, metabolites can be detected combining MS, infrared spectroscopy, ultraviolet spectroscopy, among others (Han et al., 2009; Zhang et al., 2012). In spite of the advance reached through the use of these detection methodologies, metabolomic studies still need a long period of development and improvement.

Since omics are being used, scientific world has increased the knowledge in areas where before it was utopian. Undoubtedly these techniques are powerful tools individually, but no single omics analysis can fully unravel the complexities of microbial mechanisms. In this section we review how the omics techniques were applied and how it could gain more relevance in the field of bioremediation. Integrated omics studies in other fields were previously reviewed in excellent articles (Joyce and Palsson, 2006; Steinfath et al., 2007).

### 7.2. Integrated omics to understand degradative pathways of organic compounds and heavy metals homeostasis

If cellular physiological states are defined by mRNAs, proteins, and metabolites present in a particular moment, integrated omics studies will provide a detailed picture of these states. Based on the central dogma of molecular genetics, we could hypothesize a correlation between mRNA expression levels and protein abundance. However, several studies have failed to find significant correlation between protein and mRNA abundances (Nie et al., 2007; Zhang et al., 2006), suggesting that transcriptomic and proteomic must be complemented between them. The combination of transcriptomics and metabolomics provides a powerful tool. These omics in combination are used to establish a relationship between genes/transcripts and metabolites (functional elements) in cells. In the recent years, integrated omics approaches were applied in actinobacteria. For instance, transcriptomic-metabolomic analysis in *Corynebacterium glutamicum* ATCC 13287 contributed to understand lysine metabolism (Kromer et al., 2004). In *Mycobacterium tuberculosis*, a combination of transcriptomics and proteomics was used to reveal the virulence networks of this bacterium (Gonzalo-Asensio et al., 2008). More recently, a complete integrated omics study was performed in the oleaginous bacterium *Rhodococcus opacus* PD630 (Chen et al., 2014).

Overall, studies carried out in *Streptomyces* strains were focused on setting the optimum conditions to remove pesticides and heavy metals from liquid medium or soils and optimize mixed cultures or immobilization systems. In the case of *R. jostii* RHA1 the ability of degrading toxic compounds was supported by molecular biology approaches. For instance, a two component system responsible for the transcription of five gene clusters involved in the degradation of

PCB were identified (Takeda et al., 2010). Subsequently, the regulation of phenol degradation in *R. jostii* RHA1 at transcriptional level was studied by real time PCR (Szóköel et al., 2014). Undoubtedly, the contribution of these studies to the development of more efficient bioremediation technologies will be invaluable. However, mechanisms and regulations that bacteria use during the degradation of organic compounds or to cope with heavy metals toxicity are still unknown in actinobacteria. An exception could be *A. tucumanensis* and *C. glutamicum* in which models for detoxification of copper and arsenic were proposed respectively (Dávila Costa et al., 2012; Villadangos et al., 2011). Although these models are experimentally well supported, integrated omics studies would help to prove the harmonic and synchronized function of the components of these models. For instance in *R. jostii* RHA1, label-free and redox proteomics approaches demonstrated the interaction of metabolic pathways during the accumulation of triacylglycerols (Dávila Costa et al., 2015).

### 7.3. Potential of integrated omics in co-pollution

The real worldwide problem is the co-contamination, so that bio-tools resistant to organic and inorganic toxic compounds are needed. In this sense, a key question that may be answered by integrated omics, would be: What mechanisms and regulations are behind the simultaneous resistance to these compounds in a microorganism? The relevance of this question relies on the fact that this information may be used to enhance bioremediation processes of actinobacteria through pathway-engineering techniques. Over the last years, efforts have been focused towards pathway engineering in order to enhance bioremediation of inorganic or organic pollutants. Phytochelatin and metallothioneins are metal-binding peptides produced in response to heavy metals, reducing their toxicity by sequestration. An interesting study showed that phytochelatin synthase from *Arabidopsis thaliana* overexpressed in *E. coli* resulted in 20-fold higher heavy metal accumulation (Sauge-Merle et al., 2003). Similarly, pathway engineering was used to enhance bioremediation of common organic pollutants (see Singh et al., 2008 for a comprehensive review). According to these studies pathway engineering is a successful technique, although it was never applied in bacteria resistant to both organic and inorganic toxics. In this sense, the actinobacteria *A. tucumanensis* (Albarracín et al., 2005) and *Streptomyces* sp. M7 (Benimeli et al., 2003) are excellent candidates for integrated omic studies and subsequently pathway engineering. *A. tucumanensis* is able to remove copper and degrade polycyclic aromatic hydrocarbons, while *Streptomyces* sp. M7 does the same with chromium and  $\gamma$ -HCH (Dávila Costa et al., 2011a,b; 2012; Polti et al., 2014). Undoubtedly, omics studies and pathway engineering will enable to design bioremediation processes of co-polluted environments by using these promising microorganisms.

## 8. Conclusions

The present world requires a higher production of food and an increase in industrial activity in order to satisfy needs of the population. As consequence of these anthropogenic activities, several areas contaminated with pesticides and heavy metals were detected worldwide. Bioremediation techniques have shown to be successful to deal with this concern. In this field, actinobacteria have demonstrated their potential as tools for bioremediation of several contaminants including oil, rubber, plastics, pesticides, and heavy metals, among others, based on their physiological and metabolic versatility. Significant advances in the use of actinobacteria for cleaning up processes have occurred in the last 15 years. Strategies such as bioaugmentation, biostimulation, cell immobilization,

phytoremediation, production of biosurfactants, and the use of defined mixed cultures were developed to enhance the capabilities of actinobacteria in bioremediation.

Mainly to remove pesticides, the advantages of using actinobacteria consortia were demonstrated. These advantages might be attributed to the effects of synergistic interactions among members of the association. Thus, the construction of a defined mixed culture would facilitate the examination of the characteristics of each one of its members, and the monitoring of their dynamics together. Another promising alternative is the use of immobilized actinobacteria enabling their re-utilization for bioremediation of metals as well as pesticides. The immobilization of cells for this purpose resulted in high performances, greater degradative enzymes production, and extended biotransformation reaction time. However, the application of immobilized cells to environmental area is still in its preliminary stages, although the results using actinobacteria seen so far promising.

Phytoremediation techniques, based on the interactions between plants and microorganisms, have been proposed as ecofriendly methods for cleaning up polluted soils. It has been suggested that soil microorganisms might improve pollutant mobilization and their uptake by plants. This is the case of several actinobacteria species, since members of this phylum are well distributed in the rizosphere where microbial products are enhanced. In this connection, the use of microbial products as strategy for environmental remediation could have unquestionable advantages, since it is not required that producer microorganisms have the ability to grow and survive in the contaminated sites. For instance, bioemulsifiers and biosurfactants promote desorption of a pollutant, enhancing their solubility and/or bioavailability for a microbial processing. Currently, a more pragmatic approach consists in the production of these biomolecules by actinobacteria for their subsequent isolation and application in soil washing technologies.

The real worldwide problem is co-contamination. Environments contaminated with inorganic and organic compounds are considered difficult to bioremediate since heavy metals would inhibit biodegradation of organic pollutants. Nevertheless, recent works highlighted that actinobacteria strains are able to remove heavy metals and pesticides simultaneously.

Several works reported the ability of actinobacteria to bioremediate organic compounds or heavy metals. *Streptomyces*, *Rhodococcus*, and *Amycolatopsis* are among the most studied genera, although their bioremediation skills were never supported by integrated omic approaches. The relevance of the use of omic tools relies in the fact that this information may be used to enhance bioremediation processes of actinobacteria through pathway-engineering techniques. On the basis of the existence of co-contaminated environments, engineering bio-tools resistant to organic and inorganic toxic compounds could be necessary.

In spite of the advances reached in bioremediation, most of the research concerning this issue is limited to laboratory scale studies; nevertheless the bioremediation potential of actinobacteria reviewed in the present work should be compared to *in situ* conditions to assess the success of the process. In fact, there is still a lack of information available on bioremediation technology applicable for actual field-scale treatment of soil, slurries, and liquid systems contaminated with pesticides, heavy metals or both contaminants. However, only if a deeper understanding is reached, a transfer of methods and techniques from basic research into applied science and optimized *in situ* remediation using actinobacteria will be possible.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.09.070>.

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