ROLE OF BACTERIAL AUXIN-LIKE COMPOUNDS AND SIDEROPHORES IN THE ROOT DEVELOPMENT AND HEAVY METAL UPTAKE OF SALIX VIMINALIS L. IN THE PRESENCE OF LEAD AND CHROMIUM

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ABSTRACT

A large Hungarian consortium was formed to investigate the effectiveness of phytoremediation on heavy metal polluted sites. In previous study 29 endemic bacterium strains from the same soil samples isolated and identified. A consortium of the collected bacteria could improve the root development of *Salix viminalis* L. in the presence of 100 μM Cr^{3+} and Pb^{2+}, which heavy metals inhibited the root development without the bacteria. Moreover, the bacterial consortium also changed the distribution of heavy metals in the plant tissues.

In the presented work, we tried to find the potential reasons for the changed root development and distribution of heavy metals. The production of bacterial auxin-like molecules and siderophore compounds was hypothesized and investigated. According to our results, all strains of the bacterial consortium were able to produce auxin-like and siderophore compounds. In most cases, siderophore production of bacterium strains was as effective as EDTA (ethylene diamine tetraacetate, as synthetic chelator). *Acinetobacter* strains were found to be the best siderophore and auxin-like compound producers from the applied bacteria.

INTRODUCTION

Organisms, living in the rhizosphere, can facilitate the metal uptake of plants by means of different reasons. On the one hand, bacteria and other microorganisms can produce such compounds (for example plant hormones), which have influence on the plant growth. These bacteria are so-called PGPR (plant growth promoting rhizobacteria) (McMillan, 2007). On the other hand, rhizosphere microorganisms have influence on the availability of metals by the means of production such compounds, which facilitate the metal solubility in the soil solution for example by decreasing of pH or forming complexes (Tassi, és mts., 2008).

These complex forming, chelating compounds, which are increase the availability for plants and solubility of metals in the soil, are called siderophores. Since siderophores facilitate the nutrient supplies of plants, they have an important role also in the plants growth. Furthermore, they can change the oxidation state of the metals facilitating the phytoremediation processes (Salt és mts., 1999). According to Neilands (1993), siderophores are small compounds (smaller then 1500 Da), which forming complex with Fe^{3+} through oxygen atom; in this way they can extract the Fe^{3+} from the environ and can get it into the microbial cells. Although siderophores are specific for Fe^{3+}, they can form complexes with other three valent metals (e.g. pl. Ga^{3+}, Cr^{3+} , Al^{3+}, Sc^{3+}, In^{3+}), with essential metal ions (e.g. Mg^{2+}, Mn^{2+}, Ca^{2+}) Furthermore they can form complexes with radionuclides (e.g. $Pu^{4+}$and the other actinides) (Bulman 1978).

The root system of plants and their rhizosphere microorganisms can influence together the plant development. During the interference between the plants and microorganisms, the plant growth regulators (PGR) producted by microorganisms have the most important role. The PGR compounds are such natural organic compounds, which can influence the physiological processes of plants in lower concentration then the nutrients and vitamins (Arshad és mts., 1998). The plant growth regulator effects are caused also by PGPR, which can modify the microenvironment of the root by antibiosis or antagonism, and by the PGR compounds, which are principally plant hormones, such auxins, gibberellins, cytokinins (McMillan, 2007).

MATERIALS and METHODS

The applied rhizosphere bacteria

From the rhizosphere of Salix sp. grown on HM contaminated site (Mártély, Hungary) twenty nine bacterium strains were isolated during previous study of Bay Zoltán Foundation for Applied Research Institute for Biotechnology (BAYBIO). In the presence of the consortium of these bacteria, willow cutting were able to develop roots even in the presence of toxic concentration of lead and chromium. The member of the consortium belonged to the Pseudomonaceae, Bacillaceae and Enterobacteriaceae families.

Enrichment of the bacterial consortium

The bacterial strains were maintained on Bouillon agar and they were enriched in Bouillon medium at 37°C for 48h. These single bacterial cultures were utilized for investigation of siderophore and auxin production, antagonism. These single cultures were mixed to make a consortium and they were breeding together at 37°C for 48h to make a bacterial consortium. This consortium was utilized for the investigating and heavy metal tolerance, of root development of willow cuttings in the presence or absence of rhizosphere bacterial consortium and/or Cr^{3+}, Pb^{2+} and Zn^{2+} for the determination of siderophore and auxin production. During the root development experiment, Hoogland medium was applied in 10-fold dilution in the presence or absence of 100 μM. $CrCl_3$, $Pb(NO_3)_2$ or $ZnSO_4$.

Determination of heavy metal tolerance

The heavy metal tolerance of the bacterial consortium was determined by turbidimetric methods with Hach Lange DR 5000 spectrophotometer at 600nm.

Investigation of siderophore production

The produced siderophores of the applied bacterium strains were detected by O-CAS (overlaid Chrome azurol S) method by Pérez-Miranda et al. (2007) modifying the HDTMA content of the CAS agar to 10%.

The amount of produced siderophores of the applied bacterium strains and their consortium during the root development experiment was measured by ferric perchlorate photometric method (Calvente et al., 2001). The absorbance was measured at 305 nm with Hach Lange DR 5000 spectrophotometer. The siderophore production was correlated to the chelating capacity of EDTA.

Investigation of auxin production

The auxin produced by the applied bacterium strains and their consortium during the root development experiment was measured by (Glickmann et al., 1994). The auxin content was referred to indole acetic acid (IAA).

RESULTS

Based on previous study, the consortium of the applied rhizosphere bacterium strains had effect on the root development and heavy metal uptake, which was thought to be caused by bacterial siderophore and auxin production. First the antagonistic effect between the rhizosphere bacterium strains, and their heavy metal tolerance were investigated.
Based on these investigations, antagonistic effect is not typical between the applied rhizosphere bacteria, it was detected only in some cases in fairly low degree.
Both Cr^{3+}, Pb^{2+} and Zn^{2+} increased the proliferation of the rhizosphere bacterial consortium in the presence of 0,1 mM heavy metal, which Cr^{3+} and Pb^{2+} concentration was totally toxic for the willow cuttings during the root development experiment. Although, 0,5 mM Pb^{2+} and Zn^{2+} decreased the viability, till Cr^{3+} had inhibitory effect only in 3 or more mM concentration for the bacterial consortium. The applied heavy metal concentration could not inhibited totally the bacterial proliferation, consequently some strains could tolerate even the higher heavy metal concentrations as well.
The results of siderophore investigations showed that all bacterial strains of the rhizosphere bacterial consortium could produce siderophores. Moreover, based on the quantitative measurements, the siderophores produced by the bacteria could chelating such amount of Fe^{3+} as 1,5-7 mM EDTA. This amounts was similar to the siderophore production of microorganisms (*Aspergillus niger, Bacillus cereus, Staphylococcus aure*us) documented by Pérez-Miranda et al. (2007). Aspergillus strains (1525, 1527, 1528, 1539 and 1540), *Bacillus cereus* (1533), *Bacillus mycoides* (1517, 1531), *Bacillus* sp. (1538) and *Citrobacter* sp. (1521) produced by far the highest siderophore level. Furthermore, during the root development experiment, the siderophore content was higher in the bacterial treated samples, than the untreated samples (Table 1.). The environmental bacteria of the non-sterile condition presumably caused the low concentrations of siderophore in the untreated samples,.

Table 1. Produced total indolic compounds and siderophore concentrations during the root development experiment

Sample	Indolic compond (μgml^{-1})	SD	Siderophore content (mM) regarding to EDTA	SD
Control	n.d.	-	0,01	<±1%
Treated with rhizosphere bacteria	0,112	±0,095	0,02	±17%
Treated with Zn^{2+}	n.d.	-	0,06	<±1%
Treated with Zn^{2+} and rhizosphere bacteria	0,133	±0,081	0,10	±10%
Treated with Pb^{2+}	n.d.	-	0,01	<±1%
Treated with Pb^{2+} and rhizosphere bacteria	0,148	±0,004	0,08	<±1%
Treated with Cr^{3+}	n.d.	-	0,01	<±1%
Treated with Cr^{3+} and rhizosphere bacteria	0,078	±0,024	0,05	±20%

n.d.: not detected

During the root development experiment, willow cutting could not develop (or only a little) root in the presence of 100 μM Pb^{2+} and Cr^{3+}. However, in the presence of the rhizosphere bacterial consortium the willow cuttings could develop strong (even stronger than in case of the absolutely untreated control) root system in the presence of Pb^{2+} and Cr^{3+} at the given concentrations. Since this kind of root development alluded to the presence of auxin (or other plant hormones), the bacterial auxin production was also investigated by colorimetric methods. According to the results, all bacterial strains of the rhizosphere bacterial consortium could produce auxin or a sort of indolic compounds. Regarding to the auxin production, also the *Acinetobacter* sp. strains seemed to the best. During the root development experiment, the total amount of indolic compounds was also measured and indolic compounds only in the bacterial treated samples could be detected in approximately 0,1 μgml^{-1} concentration (Table 1.). This concentration is not high, however according to Kerk et al. (2000), maybe enough to have some effect on root development. Although, lead and chromium inhibited the root development of the plants, the consortium of rhizosphere bacteria of willow was able to supply auxin-like compounds and plants could develop roots.

CONCLUSIONS

- Consequently, antagonistic effect is not typical between the applied rhizosphere bacteria.
- The applied heavy metals in 0,1 mM concentration, which concentration was toxic for the willow cuttings, had a little positive effect on the bacterial proliferation.
- The consortium of the applied rhizosphere bacterium strains had effect on the root development and heavy metal uptake, which could be caused by bacterial siderophore and auxin production. According to our results all applied bacterium strains could produce siderophore and auxin like compounds in such concentration, which could have influenced the heavy metal uptake and the root development in the presence of 100 μM Cr^{3+}, Pb^{2+} and Zn^{2+}.

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