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Short note - Nota breve

Microbial characterization of soils contaminated with heavy metals subject to phytoremediation using *Populus x canadensis*

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RIASSUNTO - *Caratterizzazione microbiologica di suoli inquinati da metalli pesanti dopo phytoremediation realizzata con Populus x canadensis* - Piante di *Populus x canadensis* appartenenti allo stesso clone (I-214), cresciute in pieno campo su un suolo contaminato da rame e zinco, hanno mostrato differenze di crescita, indipendentemente dalla concentrazione dei metalli. Ipotizzando un ruolo della componente microbica, è stata analizzata la composizione della microflora del suolo prossimo alle radici di pioppi di grandi (I-214G) e piccole (I-214P) dimensioni e del suolo non piantumato (Suolo), usando tecniche coltura-dipendenti ed -indipendenti. Tra i batteri coltivabili, Variovorax paradoxus e Chryseobacterium soldanellicola erano specie selezionate dalla presenza del pioppo. Comunità microbiche più numerose, attive e con maggiore biodiversità sono state rilevate in I-214G.

Key words: polluted soil, metabolic profile, enzymatic activities, microbial community structure, DGGE, Piedmont (NW Italy)
Parole chiave: suolo contaminato, profilo metabolico, attività enzimatiche, struttura della comunità microbica, DGGE, Piemonte (Italia)

1. INTRODUCTION

Heavy metal pollution is one of the major problems negatively affecting both human and environmental health. Sources of heavy metal pollution may be atmospheric or terrestrial and include mining, metal working industries, combustion of fossil fuels, disposal of ash residues from coal combustion, vehicular traffic and use of pesticides and fertilizers in agriculture (Clemens 2006). In order to avoid the toxicity associated to these metals, several technologies and methods have been developed to remove them from polluted soils. The use of the plants for environmental cleanup, called "phytoremediation" (Salt *et al*. 1995) is a clean and cost-effective alternative to the traditional physical-chemical methods of remediation. In addition, phytoremediation is likely to be readily accepted by a concerned public. Poplar clones able to accumulate heavy metals and cultivated in a soil contaminated by copper and zinc showed different growth rates. In order to assess the possible role of the microflora in these heterogeneous plant growth, the microbial communities structure and activity of rhizospheric, as well as bulk, soil were studied by coupling traditional culture-dependent and independent (Denaturing Gradient Gel Electrophoresis - DGGE) techniques. In addition, the rhizospheric and bulk soils were characterized through physical-chemical analysis.

2. METHODS

All the following analyses were performed on the rhizosphere of poplar with large (I-214G) and small size (I-214P), and on the bulk soil (Soil). Chemical-physical characterization of the soil samples were performed as described in Gazzetta Ufficiale Repubblica Italiana Supplemento Ordinario n° 248, 21/10/1999. Microbiological culture-dependent analyses (bacterial density, composition and structure evaluation, soil microflora carbon utilization pattern) were performed as described by Avidano *et al*. (2005), while the soil activity was evaluated through fluorescein diacetate hydrolysis (Adam & Duncan 2001).

The DNA was extracted in triplicate from the whole community of culturable bacteria and directly from the soils by using the UltraClean™ Soil DNA Kit (MO BIO Laboratories, Inc.). For DGGE analysis of the Eubacterial community, V6-V8 regions of 16S rDNA were amplified

with primers GC986f and Uni1401r as described by Felske *et al*. (1998). DGGE gels were analyzed by GelCompare II software, using UPGMA (Unweighted Pair-Group Arithmetic Mathematical Average) and Dice's coefficient.

3. RESULTS

All the samples were sandy loam soils showing similar organic carbon content, pH and amount of Cu and Zn. On the contrary, the ratio between the available and total phosphorus increased from the bulk soil (0.008%), to I-214P (0.017%) and I-214G (0.041%). The densities and the biodiversity of culturable bacteria and the activity of the microflora were lower in the bulk soil than in the rhizospheric ones. The structure of culturable microbial communities differed among the three sampling areas: the genus *Arthrobacter* was dominant in bulk soil, while the specie *Variovorax paradoxus* was prevalent in the samples I-214P and I-214G (Tab. 1).

DGGE analysis of Eubacterial and culturable bacteria communities showed low similarity among the sites. The culturable fraction and the whole Eubacterial community clustered separately. In addition, the culturable fraction and the whole Eubacteria community of the bulk soil showed the lowest biodiversity (Tab. 2). Band clonings and sequencing are in progress.

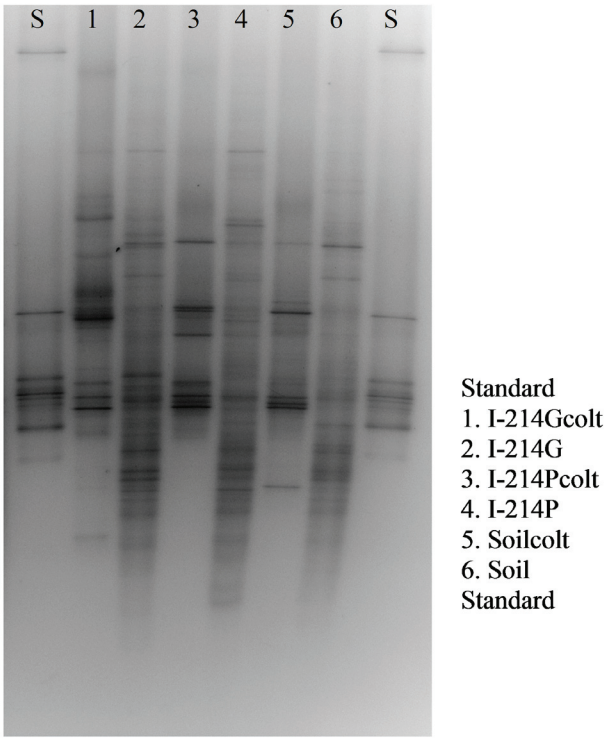

Fig. 1 - DGGE banding pattern and cluster analysis of culturable Eubacterial 16SrDNA (1, 3 and 5) and total Eubacterial 16S rDNA (2, 4 and 6).
Fig. 1 - Profili DGGE ed analisi cluster dell'rDNA 16S degli Eubatteri coltivabili (1, 3 e 5) e dell'rDNA 16S degli Eubatteri totali (2, 4 e 6).

Tab. 1 - Frequence of culturable bacterial species identified through sequencing in the three sites.
Tab. 1 - Percentuale delle specie batteriche coltivabili identificate mediante sequenziamento nei tre campioni di suolo.

	Soil	I-214P	I-214G
Bacillus sp.	3.57	3.57	0.00
Bacillus simplex	3.57	3.57	7.69
Paenibacillus lautus	7.14	10.71	0.00
Bacillus mycoides	10.71	3.57	3.85
Bacillus cereus/thuringiensis	7.14	7.14	0.00
Bacillus pumilus	3.57	0.00	0.00
Bacillus sphaericus	0.00	3.57	0.00
Bacillus subtilis	0.00	0.00	3.85
Chryseobacterium soldanellicola	0.00	10.71	11.54
Chitinophaga ginsengisegetis	0.00	3.57	0.00
Flavobacterium sp.	0.00	0.00	15.38
Flavobacterium johnsoniae	0.00	3.57	0.00
Flavobacterium columnare	0.00	0.00	15.38
Arthrobacter	46.43	17.86	7.69
Arthrobacter dextranolyticus	0.00	0.00	3.85
Streptomyces sp. TSC5	0.00	0.00	3.85
Streptomyces sp. 3674	3.57	0.00	0.00
Streptomyces canus	3.57	0.00	0.00
Streptomyces globisporum	3.57	0.00	0.00
Streptomyces recifensis	3.57	0.00	0.00
Streptomyces diastatochromogenes subsp. *luteus*	3.57	0.00	0.00
Streptomyces sp. VTT E-052902	0.00	3.57	0.00
Streptomyces sp. 3647	0.00	3.57	0.00
Variovorax paradoxus	0.00	25.00	26.92

Tab. 2 - Biodiversity indices (Shannon-Wiener, Simpson, Pielou).
Tab. 2 - Indici di biodiversità (Shannon-Wiener, Simpson, Pielou).

Sito	Shannon-Wiener	Simpson	Pielou
Soil	2.6096	0.0920	0.9211
Soil colt.	2.1169	0.1428	0.8828
I-214G	2.9547	0.0589	0.9423
I-214G colt.	2.5332	0.1004	0.8764
I-214P	3.1419	0.0486	0.9533
I-214P colt.	2.2443	0.1176	0.9360

4. DISCUSSION

The rhizospheric soils showed the higher microbial activity of culturable fraction. Culturable Gram positive bacteria were predominant in the bulk soil, while *Variovorax* was the characteristic species of poplar rhizosphere, selected by the plant itself.

Based on DGGE profiles of culturale bacterial fraction, the site I-214G showed the highest biodiversity. On the contrary, the site I-214P was characterized by the highest biodiversity of the whole Eubacterial community. Since soils showed comparable physical-chemical characteristics, but different value of the fraction of available P, the differences observed in plant growth could be ascribed to the diverse microbial community activity and structure highlighted by this work. In conclusion, in a phytoremediation project, where the plant development is relevant, it's necessary to consider the role of the microflora associated to the roots.

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