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Isolation and selection of Plant Growth Promoting Rhizobacterium strain for the decontamination of polluted soils

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The environmental contamination due to bioaccumulation in the soil of persistent chemicals such as polycyclic aromatic hydrocarbons (PAHs) is a problem that is becoming increasingly important in the world. The technologies based on the use of microorganisms able to degrade the xenobiotic substances, allow to accelerate the natural detoxification processes in the soil environment and represent an alternative method of environmental remediation compared to the harmful traditional methods (incineration and chemical treatment). For this aim we isolated, characterized and selected 14 indigenous bacteria strains obtained from soil of the industrial site ex-ACNA (Associate National Chemical Companies) in Cengio (Savona, Italy) contaminated by different classes of organic compounds (PAHs). The isolation was carried out using a minimal selective solid medium containing 0.5% of Contaminated Soil Aqueous Extract (CSAE) extracted from the soil mentioned above, with or without addition of natural nutrients (1% of soil extract obtained from freshly collected meadow soil). Further biotechnological selection allowed to detect a bacterial strain able to grow in solid medium containing 1.5% of CSAE as sole nutrient source. This new strain was identified by phenotypic (morphological and biochemical tests) and molecular methods (16S rDNAs sequence analysis of ribosomal genes) as belonging to *Methylobacterium* spp. (97%). This strain was also characterized for plant growth stimulating activities since *Methylobacterium* genus is known to improve seed germination and plant development.

The ability of *Methylobacterium* spp. strain to grow in the highly contaminated habitat was enhanced using serially enrichment strategy in liquid media containing increasing concentrations of CSAE (up to 40%), with or without addition of nutrients (1% soil extract obtained from meadow soil). During enrichment experiments, the growth of the bacteria in the liquid culture was determined by spread plate count method using minimal solid media containing the same amount of CSAE. Moreover, to demonstrate the degradative capacities of *Methylobacterium* spp. strain, GC-MS analysis of the centrifuged broth cultures supernatant containing 40% of CSAE, was performed. *Methylobacterium* spp. was able to remove partially or completely some pollutants from liquid medium containing CSAE. These results demonstrate the effectiveness of selective ecological strategy that employs indigenous strains naturally present in highly contaminated soils, able to express their potential biodegradation of xenobiotic organic compounds of industrial origin and their potential use to remediate contaminated soils.

Parole chiave: PAHs-degrading bacteria; *Methylobacterium* spp.; Plant Growth Promoting activity; bioremediation

Selected microbial strains isolated from a contaminated site as degraders of polycyclic aromatic hydrocarbons in soil bioaugmentation

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The increasing industrial development and urbanization have led to a significant degradation of the environment through release of toxic and hazardous pollutants. Polycyclic aromatic hydrocarbons (PAHs) are a major class of such persistent organic pollutants, posing serious threat to terrestrial and aquatic ecosystems due to their intrinsic low aqueous solubility, higher binding affinity toward soil organic matter, and chemical stability. PAHs may be present at high concentrations at industrial sites associated with petrole, coal tar, gas production and wood preservation industries. The ACNA site, an industrial area of Cengio near Savona, is largely contaminated by different classes of organic compounds and it has been included in the list of national priorities for environmental reclamation. As compared to the physical-chemical treatments, the use of microbial technology to clean up contaminated soils was found to be more efficient, economical and eco-friendly. The aim of this work was to assess the capacity of selected microbial strains, isolated from very same polluted soil of the ACNA site, to degrade and remove the anthropogenic organic compounds present in the aqueous extracts from an ACNA soil.

In the extraction experiments, 30 g of soil were kept under reflux in a Soxhlet with 225 mL of an acetone/n-hexane (1:1) mixture for 48 hours. The organic extracts were first dried in a roto-evaporator at 40°C and redissolved in 5 mL of acetone and 145 mL of ultrapure water (final volume of 150 mL). An aliquot of these aqueous extracts was purified by solid phase extraction (SPE) through elution in Bond-Elut C-18 cartridges. Preliminary tests were carried out to optimize the volume of extract to apply and the type of organic solvent to be used for elution. Finally, an amount of 20 mL of extract and 30 mL of organic solvents (10 mL of n-hexane, 10 mL of diethyl ether followed by 10 mL of acetone) were utilized. After extraction with C-18 cartridges, the samples were dried, redissolved with 1 mL of CH_2Cl_2, containing 100 μg/ml octafluoronaphtalene solution in CH_2Cl_2 as internal standard, and analyzed by GC-MS. The aqueous extracts from the contaminated soil (CSAE) were used at a concentration 40% as growth media of microorganisms, represented by bacteria and mould strains isolated form the same ACNA soil, in order to evaluate by GC-MS analyses the removal of polycyclic aromatic hydrocarbons (PAHs) present in the CSAE.
The results obtained both with bacteria and mould selected strains showed a significant removal of the identified pollutants that varied from 28 to 100% depending on the pollutant class. The development and optimization of such extractive, degradative and analytical methodology will allow to assess the effective ability of the isolated microbial strains to be further used in bio and mycoremediation of soils polluted by PAHs and other aromatic compounds.

Parole chiave: PAHs; chemical characterization; bioremediation