PGP ENDOPHYTIC BACTERIA AS RESOURCES FOR RHIZO-REMEDIATION OF PROTECTED AREAS AFFECTED BY PETROLEUM HYDROCARBONS

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ABSTRACT

Background information

Worldwide environmental contamination by petroleum hydrocarbons represents one of the most serious ecological problems. Soil and groundwater contamination may be caused by loss of oil from oil refineries, pipelines, and service stations. During the years physical and chemical techniques showed some drawbacks, as slow progress, high cost and inefficient removal of contaminants. Therefore, bioremediation could be considered as an acceptable solution. This technique exploits microorganisms, fungi, or plants, to eliminate or reduce the concentration of contaminants in the environment. Different microorganisms are responsible for the degradation of hydrocarbons in aerobic conditions. The enzymes involve in these processes are oxygenase such as alkane hydroxylase (*alkB*), and toluene-benzene monooxygenase (*tbmD*). Furthermore, the combined use of plants and microorganisms (i.e., phytoremediation) can be considered as a promising strategy in a decontamination process.

Main results

The sampling site is located in a regional park in Northern Italy. The whole area is considered a Site of Community Importance and belongs to the European Natura 2000 network of protected areas (SCI, European Commission Habitat Directive 92/43/EEC). In 2015, the fields and groundwaters of the park were interested by a fuel discharged, caused by an intentional damage to the gasoline pipeline crossing the area. From the spill point, due to the pendency of the site, a plume of contamination flowed south-west into the aquifer finally reaching a swampy area.

Soils samples were taken from the point of spill (SB1), a contaminated spring (SB2), the shore of the spring (SA), a grassland area (SB3) and from a swampland (SW), where moreover three plants belonging to the species *Carex pseudocyperus* L. were sampled.

The present work aimed to select and quantify the presence of hydrocarbon degrading bacteria in the sampled soils and plants in order to envisage a possible exploitation in oil phytoremediation of the natural park. The microbials analysis were performed on the bulk, rhizosphere, rhizoplane and endosphere fractions.

Firstly, the soils were characterized in order to define their texture, pH, total organic carbon (TOC), organic matter (OM), cation exchange capacity (CEC), total nitrogen, total and active limestone, bioavailable phosphorous, macro- and micro-elements and exchangeable bases using an ICP-MS. Through a gas-chromatography mass spectrometry (GC-MS) analysis were defined the concentrations of Total Petroleum Hydrocarbon C>12 and C≤12. In almost all the samples concentrations of C>12 and C≤12 hydrocarbons and Zn exceeded law limits (D.Lgs. 152/2006 and Directive 2000/60/EC).

Total bacterial 16S rRNA gene, *tbmD* gene and *alkB* gene present in environmental DNA of soils (SB1, SA, SB2, SB3, BULK) and of rhizosphere (RS) and rhizoplane (RP) of *Carex pseudocyperus* plant were quantified by real time qPCR. Total bacteria were between 10^3 gene copies g dw^{-1} in SB2 and 10^7 gene copies g dw^{-1} in RS and SA. The results obtained on the quantification of the *tbmD* gene were between 10^5 and 10^9 gene copies g dw^{-1}, detected respectively in SB2 and SA. The quantification of the *alkB* gene was between 10^5 and 10^8 gene copies g dw^{-1}, found respectively in SB1 and SB2, SA and RS.

The evaluation of the natural bioattenuation of ethylbenzene, C>12 and C≤12 hydrocarbons and a mixture of *o*-, *m*- and *p*-xylenes, was carried out with soil slurries set up on samples SB1, SA, SB2, SB3. The

biodegradation potential of ethylbenzene and xylenes was between 32% and 68.8%, in all the samples. Hydrocarbons C≤12 were degraded in the range of 35% and 63% in samples SB1 and SB3. The class of hydrocarbons C>12 was degraded with good (57%) and discrete (32%) rate in SB2 and SB3.

Enumeration and isolation of viable heterotrophic bacteria was performed by plate count method for sieved bulk, rhizosphere soil and for plant endosphere.

All the isolated bacterial strains were able to growth on 400 ppm hexadecane as sole carbon and energy source. Moreover, they were also tested for exadecane-adesion capacity, according to Abdulla *et al.* (2014). The SB1 strains were 100% positive to the test, SA 67%, SB3 71%, BULK 50%, RS 67% and Endophytes 75%.

In view of an assisted phytoremediation intervention, bacterial strains isolated from samples SA, BULK, RS and EN were tested for their capacity to promote the plant growth (i.e., plant growth promoting, PGP). Among all, only rhizosphere endophytic bacteria isolated from *C. pseudocyperus* were positive to each PGP tests. Rhizosphere endophytes were tested for Zn resistance, resulting in 27% of strains being able to grow in the presence of 0.5 mM $ZnSO_4$. Isolated strains were categorized by ITS profiling and subsequently identified by 16S rRNA gene sequencing. The most abundant species were: *Bacillus mobilis, Micrococcus luteus, Planomicrobium glaciei* and *Pseudomonas koreensis*. In growth pouches experiments set-up to prime *Zea Mais* and *Brassica napus* seeds, two endophytic and one shore spring strains used to prime the seeds determined a considerable increase in plant biomass after 28 day incubation.

Conclusions

The abundance of functional genes such as *tbmD* e *alkB* codifying for aromatic and aliphatic hydrocarbon oxygenases and the presence of bacterial population able to degrade hydrocarbons indicate that a natural bio-attenuation process is feasible at the site. In order to speed up the process, a phytoremediation intervention, in line with the park destination of the area, is highly recommended and supported by the association of PGP rhizosphere endophytes to plants thus promoting hydrocarbon biodegradation and zinc uptake from soil.

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