[E.30]

Biomonitoring of chlorinated solvents-degrading bacteria in contaminated soil and groundwater

Simona Rossetti[1,*], Bruna Matturro[1], Federico Aulenta[2], Francesco Valentino[2], Marco Petrangeli Papini[2], Valter Tandoi[1]

[1] *Water Research Institute - CNR, Italy*
[2] *Department of Chemistry, Sapienza University of Rome, Italy*
Keywords: Biomonitoring; Bioremediation; Microbial diversity; Reductive dechlorination

The definition of the actual structure and composition of microbial communities occurring in contaminated soil and groundwater, through the application of in situ methodologies, would strongly assist the definition of the aquifer bioremediation potential. In addition to Real-time PCR approach, in situ detection methods (FISH and CARD-FISH) were also applied in this study for tracking key-dechlorinating bacteria under a wide range of dechlorination activity conditions. The abundance of *Dehalococcoides* species, known to be able to completely degrade chlorinated ethenes to harmless ethene, was estimated on groundwater samples from 19 wells at a chlorinated solvent contaminated site located in Northern Italy. They ranged between 5×10^3 and $2{,}5 \times 10^5$ cells/ml groundwater (5-35% of the total Bacteria) by FISH and were estimated as 2.5×10^3 - 3.1×10^6 16S rRNA gene copies per ml of contaminated groundwater by Real Time PCR. Furthermore, the structure of microbial communities in groundwater and in soil core samples collected at different depths (from–5 to–45 m from the site surface) and locations within the contamination plume was defined by means of additional probes for known and putative dechlorinators and for the main phyla within Bacteria and Archaea domains. The molecular characterization was also performed on microcosms set up with aquifer material collected at different soil depths and amended with a range of electron donors to evaluating the bioremediation potential of the contaminated site. The analysis was effective and allowed the abundance estimation of the active fraction of the target bacteria. Real Time PCR was indeed applied to quantify gene copies number of 16S rRNA as well as key-functional genes in DNA/RNA extracted from contaminated groundwater samples. The comparison of the results obtained by FISH and Real Time PCR showed their effectiveness for tracking key-bacteria under a wide range of environmental conditions by producing confirmatory data always recommended when complex site samples are analysed.

doi:10.1016/j.jbiotec.2010.08.126

[E.31]

Organization and dynamism of gamma-hexachlorocyclohexane-degrading bacterium *Sphingobium japonicum* UT26 genome

Yuji Nagata[1,*], Shunsuke Natsui[1], Yoshiyuki Ohtsubo[1], Natsuko Ichikawa[2], Akiho Ankai[2], Akio Oguchi[2]

[1] *Graduate School of Life Sciences, Tohoku University, Japan*
[2] *National Institute of Technology and Evaluation (NITE), Japan*
Keywords: Environmental pollutants; Bioremediation; Halogenated compounds; Sphingomonads

gamma-Hexachlorocyclohexane (gamma-HCH/gamma-BHC/lindane) is a completely man-made chlorinated pesticide that causes serious environmental problems due to its toxicity and long persistence in upland soils. *Sphingobium japonicum* UT26 utilizes gamma-HCH as a sole source of carbon and energy under aerobic conditions. The gamma-HCH degradation pathway in UT26 has been revealed, and fifteen *lin* genes involved in the gamma-HCH degradation in UT26 have been identified. The complete sequencing of UT26 genome revealed that the genome consists of two circular chromosomes with sizes of 3.5 Mb (Chr 1), 682 kb (Chr 2), a 191-kb large plasmid (pCHQ1), and two small plasmids with sizes of 32 and 5 kb. The *lin* genes are dispersed on Chr 1, Chr 2, and pCHQ1. Comparison of the UT26 genome with those of other Sphingomonadaceae strains demonstrated that the specific *lin* genes for conversion of gamma-HCH to beta-ketoadipate (*linA, linB, linC, linRED,* and *linF*) are located on the DNA regions unique to the UT26 genome, indicating that these *lin* genes were probably acquired recently. On the other hand, *linGHIJ* and *linKLMN* are located on conserved regions in the genomes of sphingomonads, suggesting that the *linGHIJ*-encoded beta-ketoadipate pathway and the LinKLMN-type ABC transporter system are involved in core functions of sphingomonads. Based on these results, we proposed a hypothesis that UT26 has been created by recruiting the specific *lin* genes into a strain having core functions of sphingomonads. Most of the specific *lin* genes in other gamma-HCH degrading strains are located on plasmids and associated with IS*6100*. Analysis of spontaneous *linA*-, *linC*-, and *linRED*-deletion mutants of UT26 revealed the involvement of IS*6100* in their deduced genome rearrangements. These facts strongly suggested that IS*6100* has important roles for the distribution of the specific *lin* genes as well as the genome rearrangements in environments.

doi:10.1016/j.jbiotec.2010.08.127

[E.32]

Improved biocatalysts for remediation of pcb-contaminated environments

M. Saavedra[1], F. Acevedo[1], V. Latorre[1,2], B. Ponce[1], M. Gonzalez[1], M. Seeger[1,*]

[1] *Universidad Tecnica Federico Santa Maria, Chile*
[2] *Universidad de Magallanes, Chile*
Keywords: PCBs; Bioremediation; Genetically modified organisms; *Burkholderia xenovorans* LB400

Polychlorobiphenyls (PCBs) are widespread toxic persistent organic pollutants. Diverse microorganisms capable of degrading PCBs have been reported. However, most aerobic PCB-degrading strains are unable to degrade chlorobenzoates (CBAs), leading to its accumulation as dead-end metabolites (Pieper and Seeger, 2008). Further incomplete degradation of CBAs by environmental microorganisms could indirectly generate toxic metabolites, such as the antibiotic protoanemonin, interfering with PCB catabolism, and constituting a stressful condition for bacteria. Oxidative stress observed during PCB-degradation also affects bacterial viability. In this report, to improve PCB-biodegradation, two different strategies had been studied. Firstly, the construction of a recombinant strain capable of degrading PCBs and CBAs. Therefore, the *bph* locus of *Burkholderia xenovorans* LB400 was incorporated into the genome of the CBA-degrader *Cupriavidus necator* JMP134-X3. The modified strain, JMS34, mineralized 3-CB, 4-CB, 2,4′-CB and 3,5-CB. Bioaugmentation of PCB-polluted soils with *C. necator* JMS34 and *B. xenovorans* LB400 was monitored. Strain JMS34 degraded, in one week, 99% of 3-CB and 4-CB and 80% of 2,4′-CB in sterile and non-sterile soil. In contrast, PCB-degradation by strain LB400 was 73% (sterile soil) and 50% (non-sterile soil). A second strategy studied to promote PCB-degradation, was to increase antioxidant activities by the construction of a recombinant strain able to overexpress oxidative stress genes and by the addition of exogenous antioxidant compounds. The recombinant strain showed