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IN VITRO VALIDATION OF A MICROARRAY DNA-CHIP FOR THE DETECTION OF DECHLORINATING BACTERIA

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Abstract

Large amounts of diverse toxic haloorganic compounds contaminate sediments, harbor sludges, soils and groundwater; there they can undergo reductive dehalogenation carried out by indigenous biomass, thus being detoxified. Reliable and fast methods for the detection and monitoring of biodegradative potential and actual catabolic activity in contaminated sites are needed in the perspective of developing biostimulation and bioaugmentation strategies.

A universal array-based approach combined with the Ligation Detection Reaction (LDR) makes use of two probes. A fluorescent labelled discriminating sequence detecting a point mutation occurring uniquely in the target microorganism. The second probe matches all the bacterial sequences of a defined target group and includes a target common oligonucleotide and a zip-code moiety. The ligation reaction solution is loaded onto an array which presents zip-code complementary sequences together with the zip-code probes and the DNA extracted from a sample. Only in the case of a perfect complementarity between the template and both the designed probes the ligation reaction occurs and fluorescence is detected. LDR microarray approach avoids preferential hybridization, cross-hybridization and presents a high discriminating potential. In addition, the presence of the zip-code allows to use the same chip with different probes thus skipping an optimization step.

Discriminating sequences and common oligonucleotides were designed on the 16S rDNA gene sequences of 18 known dechlorinating strains, belonging to different bacterial groups and each exhibiting different substrate specificity. The obtained sequences were tested *in silico* and all of them proved efficient in selectively discriminating the target sequences among the others. Finally, an *in vitro* validation of the microarray was conducted, by hybridizing on the chip the amplified 16S rDNA sequences extracted from 6 of the abovementioned target microorganisms pure cultures, namely *Desulfitobacterium chlororespirans* Co23, *Sulfurospirillum halorespirans*, *Dehalococcoides* sp. BAV-1, *Geobacter lovleyi* SZ, *Desulfomonile tiedjei* DCB-1 and *Desulfuromonas chloroethenica* TT4B. The sequences of the abovementioned microorganisms originated a strong and defined fluorescence spot signal with the respective specific probe; on the contrary no signal was obtained with the negative controls (non-target sequences).

In conclusion, even though other strain specific probes must be validated and their sensitivity determined, the proposed approach proved effective in discriminating some environmentally relevant dechlorinating microorganisms and thus promising for the development of future technologies.