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BB P03. Aerobic removal of methoxychlor contaminated soil by Streptomyces sp. A14

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Organochlorine pesticides (OP) have aroused global concern due to their long persistence, low biodegradability, wide range distribution in the environment and chronic adverse effect on wildlife and humans. Methoxychlor (MTX) is a toxic OP that was used in industrial and agricultural activities and for the malaria control. Although the use of MTX has been internationally banned it can still be detected in the environment like in the northwest of Argentina. Bioremediation offers the possibility to environment cleanup of pollutants, such as OP, using natural biological activity. However, there is little information available about microbial MTX degradation.

Actinobacteria have a great potential for bioremediation of toxic compounds, in addition strains of Streptomyces genus may be well suited for soil inoculation as a consequence of their mycelial growth habit, relatively rapid rates of growth and colonization of semi-selective substrates. In our laboratory, we isolated an actinobacteria strain from OP-contaminated soil in Santiago del Estero, Argentina, identified as Streptomyces sp. A14, able to remove and degrade MTX from liquid minimal medium. Thus, the purpose of this work is to contribute to the study of bioremediation of MTX by actinobacteria in contaminated soils. Glass pots were filled with 80 g of soil at 20% moisture. The soil samples were sterilized and the soil humidity was adjusted with sterile water and a MTX solution for different final concentrations (8.30 and 16.60 mg kg^{-1} wet weight (ww) soil). For soil samples inoculation, the microorganism was precultured in Tripticase Soya Broth with MTX (1.66 mg L^{-1}). Soil samples pots with MTX and without MTX as control, were inoculated with Streptomyces sp. A14 (2 g kg^{-1} ww soil). Soil pots were incubated at 30 ºC for 28 days. Samples were taken each 7 days. The growth was measured as CFU kg^{-1} and residual MTX from soil was determined by gas chromatography.

Our results indicate no significant differences in the growth at the different MTX concentrations added and in the control without MTX. The cell concentration increased up to 2 log units and the maximum growth of Streptomyces sp. A14 was 1.78×10^7 CFU g^{-1} ww soil, at 14 days of incubation. In both concentration assayed the microorganism was able to remove the soil pesticide, reaching the maximum removal percentages (36.14 and 76.02%) at 28 days of incubation. Finally, we suggest that Streptomyces sp. A14 has a big potential for bioremediation of soils contaminated with high MTX concentrations.

BB P04. Characterization of Pseudomonas strains able to hydrocarbon degradation and polyhydroxyalkanoates production

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Bioremediation is an attractive and environmental friendly approach for the cleanup of contaminated sites. It exploits the potential of naturally occurring microbial populations, or in some cases, introduces microorganisms with a known ability to degrade the contaminants. This technique is named bioaugmentation. Desirable characteristics in strains used in bioremediation are the high stress resistance and increased fitness. We propose that those characteristics could be conferred by the polyhydroxyalkanoates (PHA) accumulation capability. Under this context, our approach consisted in to isolate and characterize bacterial strains able to accumulate PHA from hydrocarbon contaminated environments. Two strains - Pseudomonas sp. KA-08 and Pseudomonas sp. KB-08 - were selected by their ability to grow in high amount of kerosene and diesel and to accumulate PHA from sodium octanoate as well. Comparison of the sequences and phylogenetic analysis of 16S rRNA gene allowed determine that both strains are related to P. putida. PHA was determined by gas chromatography (GC) analysis showing medium-chain length PHA, especially C8. In minimal medium with sodium octanoate as carbon source, KA-08 accumulated 15.3 ± 1.5 % of dry weight and KB-08 7.5 ± 0.8% of dry weight. We studied the type of PHA synthase present on each strain, obtaining positive results for class II phaC2 and phaC1 synthases. The comparison of nucleotide and amino acid sequences of class II synthases in both isolates showed more than 90% of similarity with sequences of Class II polyhydroxyalkanoate synthases from various Pseudomonas strains. In KB-08, a nonsense mutation was observed in phaC2 gene. Both strains were able to grow in crude oil, kerosene, diesel and xylenes as sole carbon source in high agitation conditions (300 rpm). Diesel and kerosene degradation were determined by GC. Kerosene degradation was higher than diesel's reaching 39% and 35% for KB-08 and KA-08, respectively, after 25 days of culture. No growth in hexane or octane was observed in liquid medium under high agitation condition. However, both strains were able to grow in solid media supplemented with hexane but when octane was used as sole carbon source, only KA-08 was able to grow. PCR analysis of the alkB gene showed differences between the amplicons obtained from KA-08 and KB-08 that could explain these differences.In addittion, both strains showed different results regarding xylenes mix. While KB-08 showed a good growth at 0.5% v/v, KA-08 needed at least 1% v/v to growth, perhaps because a different usage of the xylene isomers. These capabilities of these isolates regarding PHA accumulation and growth in different hydrocarbons and its degradation, will allow us to construct a rational design of bacterial consortia.