Selective enrichment of hydrocarbonoclastic bacteria producing storage compounds of biotechnological relevance

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Resume:

Hydrocarbonoclastic bacteria are important players in bioremediation of hydrocarbon contaminated ecosystems with additional potential for application in biological treatment of industrial wastewaters. Synthesis and accumulation of storage lipids such as triacylglycerols (TAGs) and wax esters (WEs), as well as polyhydroxyalkanoate (PHA) polymers, has been reported in this group of bacteria when submitted to growth-limiting conditions (e.g. nitrogen limitation). These compounds are relevant raw materials for a variety of industrial applications in biofuels and oleochemicals production. Its biosynthesis in combination with industrial wastewater treatment can contribute to make the process more economic and environmentally sustainable. The aim of this work was to obtain suitable inocula for use in biotechnological processes to produce valuable bacterial products from hydrocarbon-based wastewaters. These carbon-rich nutrient-poor wastewaters exhibited appropriated conditions to promote bacterial storage materials production, thus being an interesting application target for the proposed combined approach.

Sludge collected from the sedimentation tank of a wastewater treatment plant, located at a service station for repair and maintenance of power plant equipment, was enriched in carbon storage producing bacteria. The enrichments were carried out in 250 ml flasks with a working volume of 50 mL each, at 22ºC with shaking (150rpm). Wastewater collected from the same service station, containing lubricant and engine oil waste, was use as carbon source. The selective pressure was applied in the form of alternating periods of presence of the carbon substrate (carbon-rich and nitrogen-poor medium) followed by its absence. Cells having stored sufficiently large amounts of carbon reserve materials can survive the starvation period, whereas non-accumulating bacteria were not able to survive (selection phase). In a first period, the accumulation and selection phases had the duration of 7 days each and in a second period the accumulation phase was decreased to 3 days. The total process had the duration of 7 months.

Throughout the enrichment, biomass samples were collected and characterized in terms of lipid storage profile by thin-layer chromatography (TLC) analyses and bacterial diversity by 16S rRNA-based techniques (DGGE, cloning and sequencing).

The obtained TLC profiles showed a decrease in TAG levels with a concomitant increase in WEs levels throughout the enrichment process. Interestingly in the final phase of the enrichment it was detected an increase in the levels of a lipidic compound of unknown identity.

DGGE profiles revealed a decrease of bacterial diversity throughout time until the establishment of a nearly stable bacterial profile. The majority of bacterial 16S rRNA gene sequences retrieved from the obtained culture belonged to the phylum Proteobacteria, being mainly assigned to the orders *Pseudomonadales* and *Burkholderiales*. Some DGGE-bands corresponded to sequences with high similarity to those of members of the genus *Rhodococcus*, *Acinetobacter* and *Pseudomonas* (99% similarity) which are known for their ability to produce TAG, WEs and PHAs respectively. The remaining sequences showed also high levels of similarity (.> 95%) to those of members of the genus *Caulobacter*, *Zoogloea* and *Acidovorax*, where some species are described as capable to produce and accumulate polyhydroxybutyrates (PHBs).

The results obtained in this work show the potential of using alternating substrate availability and absence as a selective pressure to obtain a stable hydrocarbonoclastic consortium able to produce several types of lipidic storage compounds from real hydrocarbon-based wastewaters.