Screening for novel biodegraders in metagenomic libraries of petroleum-associated environments

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The major motivation of all researchers involved in biodegradation studies is, undoubtedly, the search for versatile microorganisms capable to efficiently degrade a great variety of pollutants at low operational costs. Besides, the degradation pathways of many pollutants still remain unknown or poorly characterized, due to the re-isolation of the same species when traditional cultivation methods are used to recover pollutant degrading microorganisms. Although diverse bacteria capable of degrading petroleum hydrocarbons have been isolated and characterized, the vast majority of hydrocarbon-degrading bacteria, including anaerobes, probably remain undiscovered (Harayama et al., 2004). The study and use of genomes of such uncultured microbes has become possible through metagenomics, a culture-independent molecular method which allows one to explore the metabolic potential of the uncultivated biodiversity by cloning large DNA fragments directly isolated from the environment (Handelsman *et al*., 1998). Metagenomics has been successfully applied to isolate novel biocatalysts from the uncultured microbiota from diverse environments, based mainly on functional screening assays to identify clones carrying the desired traits (Uchiyama *et al*., 2005). This is significantly relevant considering that genes coding for important metabolic functions frequently present low levels of conservation, making the comparison of clone sequences with homologous sequences available at databases very difficult. In addition, sometimes the catabolism of specific pollutants may be achieved only when two or more bacteria are grown together, each one contributing with part of the catabolic pathway, as in the case of polychlorinated biphenyls (Abraham *et al*., 2002). Considering that only 2% of the microorganisms present in the world have been evaluated for their biocatalytic potential, metagenomic studies may offer a valuable tool for the exploration of the untapped microbial diversity. Among the most significant biocatalysts isolated from metagenome, we should mention lipases/esterases, β-lactamases, proteases, nitrilases, polysaccharides-modifying enzymes (including agarases, celulases, α-amilases, xylanases, pectate liases), oxidorecdutases and dehydrogenases, enzymes involved in the biosynthesis of antibiotics and vitamins and, more recently, enzymes involved in the catabolism of aromatic hydrocarbons (Yun *et al*., 2004; Walter *et al*., 2005; Voget *et al*., 2003; Daniel, 2004; Streit & Schmitz, 2004; Ferrer *et al*., 2005; Knietsch *et al*., 2003; Song *et al*., 2005; Uchiyama *et al*., 2005; Vasconcellos *et al*., 2010). The enzymes selected from an environmental sample where the enzymatic repertoire is the result of a natural selection would reflect common activities and will be biased through the dominance of a few abundant organisms. In this respect, one of the most exciting current research endeavours is the exploration of the biological diversity that exists in the extreme conditions that occur at the margins of the biosphere and its interface with the lithosphere. Environments with extreme pH and/or temperature, low water activity, high irradiation, and so on, serve as natural reservoirs for more robust biocatalysts that in the future could become indispensable for industrial applications. In this context, accessing new catalysts from petroleum reservoirs will not only lead to the discovery of new green biocatalysts of potential use in biotechnological processes, such as bioremediation and microbial enhancement oil recovery (MEOR), but will provide a more comprehensive view of the poorly understood biodegradation processes that take place in such hostile environments.