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PROTEOMIC ANALYSIS OF DEGRADATION PATHWAYS IN *RHODOCOCCUS* SP. STRAIN TFB

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Some important microorganisms in biodegradation are refractory to genetic manipulation being necessary to use different approaches to study the metabolic pathways involved in the catabolism of pollutants. *Rhodococcus* sp. strain TFB is a versatile Gram positive bacterium able to grow on a wide variety of contaminant compounds as carbon and energy sources. Proteomic analysis has been used to study the metabolic pathways involved in the catabolism of such compounds identifying most of the differentially induced proteins in 2D-DIGE experiments. Reverse genetics has been applied to clone the corresponding genes. A cluster of structural genes involved on phthalate degradation to protocatechuate plus a divergently transcribed gene for an IclR-type regulator, were localised in a genomic library of TFB (Tomás-Gallardo et al., 2006). The *pht* operon is inducible by phthalate and does not show catabolite repression by glucose. A cluster of genes, similar to those previously described in *Sphingomonas macrogolitabida* strain TFA (Martínez-Pérez et al., 2004) for tetralin catabolism, have been identified in TFB. Those genes are organised in three operons, which are induced by either tetralin or naphthalene, and are subjected to catabolite repression by glucose. Transcription start points of two operons have been located using a primer extension assay. Promoter sequences have been characterized using translational fusions in a *Rhodococcus-E.coli* promoter-probe vector. DNA-Protein interactions have been tested using biotinilated DNA bound to Streptavidin Magnetic Beads (Dynabeads, Invitrogen).

Thn proteins are also induced with naphthalene, but a Gentisate dioxygenase is only induced in naphthalene-grown cells. Reverse genetic is being applied for the cloning of gentisate degradation genes. Salicilaldehyde dehydrogenase and Gentisate dioxygenase activities have been tested total crude extract of TFB tetralin-, naphthalene-, salicylate-, and glucose-grown cell.