Cloning and functional analysis of the genes coding for 4-Aminobenzenesulfonate 3,4-dioxygenase from hydrogenophaga Sp Pbc

Abstract

The gene coding for the oxygenase component, sadA, of 4-aminobenzenesulfonate (4-ABS) 3,4-dioxygenase in Hydrogenophaga sp. PBC was previously identified via transposon mutagenesis. Expression of wild-type sadA in trans restored the ability of the sadA mutant to grow on 4-ABS. The inclusion of sadB and sadD, coding for a putative glutamine-synthetase-like protein and a plant-type ferredoxin, respectively, further improved the efficiency of 4-ABS degradation. Transcription analysis using the gfp promoter probe plasmid showed that sadABD was expressed during growth on 4-ABS and 4-sulfocatechol. Heterologous expression of sadABD in Escherichia coli led to the biotransformation of 4-ABS to a metabolite which shared a similar retention time and UV/vis profile with 4-sulfocatechol. The putative reductase gene sadC was isolated via degenerate PCR and expression of sadC and sadABD in E. coli led to maximal 4-ABS biotransformation. In E. coli, the deletion of sadB completely eliminated dioxygenase activity while the deletion of sadC or sadD led to a decrease in dioxygenase activity. Phylogenetic analysis of SadB showed that it is closely related to the glutamine-synthetase-like proteins involved in the aniline degradation pathway. This is the first discovery, to our knowledge, of the functional genetic components for 4-ABS aromatic ring hydroxylation in the bacterial domain.