Research Report

Characterization of the Decolorizing Activity of Azo Dyes by *Bacillus subtilis* Azoreductase AzoR1

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ABSTRACT

Azo dyes are synthetic organic colorants which are regarded as pollutants once they are released into the environment. Azoreductase, azo dye-degrading enzyme, can transform azo dye into colorless compounds by reductive cleavage of the azo bond. Recent study uncovered the regulation of *azoR1* expression, which negatively controlled by YodB repressor, in soil bacterium, *B. subtilis*. The level of *azoR1* transcription and AzoR1 protein were increased in *yodB* mutant. In this study, the ability of reducing azo dyes by *B. subtilis* strain ORB7106 (*yodB* mutant) was studied. Characterization of four azo dyes (Azobenzene, Methyl Red, Orange G and Congo Red) degradation activity was investigated. In comparison, the decolorization efficacy of ORB7106 was significantly better than the wild type strain (JH642). ORB7106 showed 50-98% decolorization during 48 hr. The bacterium exhibited a remarkable color removal capability over a wide range of dye concentrations (10-200 mg/L), pH (5-9) and temperatures (25-45°C). The reduction of azo dyes was due to adsorption to bacterial cells and/or to degradation. An oxygen insensitive azoreductase gene, *azoR1*, was amplified by polymerase chain reaction (PCR) from the genomic DNA of JH642 then cloned into pET-16b overexpression vector, and yielded 84% positive clones by screening using colony PCR. The *azoR1* sequence in the recombinant plasmid was verified by DNA sequencing and designated as pHis-AzoR1-3. The recombinant azoreductase is now ready for expression in *E. coli* for further enzyme characterization.