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Gene Cloning and Expression of Azoreductases from Azo-Degraders Lysinibacillus macrolides and Bacillus coagulans Isolated from Egyptian Industrial Wastewater

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Abstract : Textile industry is one of the important industries in the worldwide. It is known that the eco-friendly industrial and agricultural activities are significant for socio-economic stability of all countries. The absence of appropriate industrial waste water treatments is essential barrier for sustainable development in food and agricultural sectors especially in developing country like Egypt. Thus, the development of enzymatic bioremediation technology for textile dye removal will enhance the collaboration between scientists who develop the technology and industry where this technology will be implemented towards the safe disposal of the textile dye wastes. Highly efficient microorganisms are of most importance in developing and using highly effective biological treatment processes. Bacterial degradation of azo dyes is generally initiated by an enzymatic step that involves cleavage of azo linkages, usually with the aid of an azoreductase as electron donor. Thus, expanding the spectrum of microorganisms with high enzymatic activities as azoreductases and discovering novel azo-dye degrading enzymes, with enhanced stability and superior catalytic properties, are necessary for many environmental and industrial applications. Consequently, the use of molecular tools has become increasingly integrated into the understanding of enzyme properties and characterization. Researchers have utilized a gene cloning and expression methods as a tool to produce recombinant protein for decolorizing dyes more efficiently. Thus, presumptive evidence for the presence of genes encoding azoreductases in the genomes of selected local, and most potent azo-degrading strains were obtained by using specific oligonucleotides primers. These potent strains have been isolated from textile industrial wastewater in Egypt and identified using 16S rRNA sequence analysis as 'Lysinibacillus macrolidesB8, Brevibacillus parabrevisB11, Bacillus coagulansB7, and B. cereusB5'. PCR products of two full length genes designated as (AZO1;621bp and AZO2;534bp) were detected. BLASTx results indicated that AZO1 gene was corresponding to predicted azoreductase from of Bacillus sp. ABP14, complete genome, multispecies azoreductase [Bacillus], It was submitted to the gene bank by an accession no., BankIt2085371 AZO1 MG923210 (621bp; 207 amino acids). AZO1 was generated from the DNA of our identified strains Lysinibacillus macrolidesB8. On the other hand, AZO2 gene was corresponding to a predicted azoreductase from Bacillus cereus strain S2-8. Gene bank accession no. was BankIt2085839 AZO2 MG932081 (534bp;178 amino acids) and it was amplified from our Bacillus coagulansB7. Both genes were successfully cloned into pCR2.1TOPO (Invitrogen) and in pET28b+ vectors, then they transformed into E. coli DH5α and BL21(DE3) cells for heterologous expression studies. Our recombinant azoreductases (AZO1&AZO2) exhibited potential enzyme activity and efficiently decolorized an azo dye (Direct violet). They exhibited pH stability between 6 and 8 with optimum temperature up to 60°C and 37 °C after induction by 1mM and 1.5mM IPTG, for both AZO1 &AZO2, respectively. These results suggested that further optimization and purification of these recombinant proteins by using different heterologous expression systems will give great potential for the sustainable utilization of these recombinant enzymes in several industrial applications especially in wastewater treatments.

Keywords : azoreductases, decolorization, enzyme activity, gene cloning and expression
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