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Utilization of agro/industrial wastes for high-level expression of recombinant endo-mannanase by *Escherichia coli* and its application in oil extraction from Copra

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The high enzyme titers of recombinant endo-mannanases (EC 3.2.1.78) have really boosted their industrial prospects in food, feed, oil drilling, textiles and pulp sectors but the high cost associated with their production is still acting as a major deterrent. In the present work, three pronged-approach was employed to ameliorate and economize ManB-1601 (recombinant endo-mannanase, accession no: KM404299) production. Firstly, pRSETA manb-1601 construct was transformed in HI-Control *E.coli* BL21 (DE3) cells which resulted in 2.7 fold (1821 IU/ml, 42.5 hours post-induction) increase in ManB-1601 production as compared to the previous host. The second approach focused on understanding the interplay between various nutritional and physical factors. Shifting of induction time to 5 hours resulted in up to 1.89 fold improvement in enzyme yield (2268 IU/ml) and volumetric enzyme productivity (81 IU/ml/h). Replacement of IPTG with lactose improved ManB-1601 production by 1.77 fold (4031 IU/ml). Efforts to improve the cell membrane permeability using chemical (glycine, calcium chloride) and physical [incubation temperature higher (42o C) or lower (16-25o C)] methods were found counter-productive. Increment in protein (by peptone) and carbohydrate (by glycerol) concentration to 2.0 % (w/v) and 0.25% (v/v) resulted in up to 2.08 fold (8406 IU/ml) increase in ManB-1601 titers. However, doubling (2X) or quadrupling (4X) the concentration of optimized medium proved detrimental. Under the third approach, up to 70.45% (w/v) of protein in the optimized media was successfully replaced with soluble protein from defatted flax seed meal in presence of glycerol (0.25% v/v) and lactose (1.5 mM) and resulted in 5926 IU/ml of ManB-1601. Treatment of grated copra with purified ManB-1601 (220 IU/g) resulted in 9.1% higher oil yield when compared to control. Laser scanning confocal microscopy and scanning electron micrographs studies depicted significant reduction in oil-droplet size and subtle changes in the copra morphology, respectively in enzyme treated samples. No change in FAME profiles was observed after ManB-1601 treatment.

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Mycodegradation of azo dyes and enzyme profiling

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One of the major chemical pollutants from textile industries is the toxic azo dyes and their reductive cleavage products that can be carcinogenic and even mutagenic. Large volume of azo dye-containing wastewater gets discharged into the natural ecosystem and poses serious health hazard to humans and aquatic life. Various physicochemical and biological methods have been developed for the reduction of azo dyes to achieve decolorization. Biological methods based on utilization of ligninolytic fungi are widely researched. The present study was aimed towards degradation of azo dyes viz. congo red and amido black 10B. Dye contaminated soil samples were collected and screened for obtaining potential fungal strains for dye degradation. Two potential fungal strains were isolated and identified as *Rhizoctonia* and *Mucor* on the basis of morphological and microscopic observations. *Rhizoctonia* sp. showed 94% decolorization of congo red within 60 hours, while *Mucor* sp. exhibited 60 % decolorization of amido black 10B following 180 hours of incubation. Enzyme profiling detected notable levels of both laccase and manganese peroxidase during decolorization. Moreover, manganese peroxidase appeared to be the predominant enzyme responsible for dye decolorization (744.6 U ml⁻¹, congo red; 74.46 U ml⁻¹, amido black 10B). The isolated fungal strains exhibited adequate potential to decolorize the azo dyes. The strains can provide an eco-friendly and cost effective tools for bioremediation.

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