

Phylogenetic Identification of Laccase Producer Bacteria Isolated From Pulp and Paper Industry Weastwater

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Background & Objectives: Complex of enzymes in the name of Ligninase are involved in lignin degradation. Special properties of Laccase among the lignin degradation enzymes make them more important. Oxidation of wide range substrates, high heat tolerance and acidic isoelectric pH are special properties of laccase. In this research our aim was isolation and identification of laccase producing microorganism from weastwater.

Methods: Laccase producing bacteria were screened from pulp and paper industry weastwater. Bacteria from collected samples were isolated and purified on Nutrient agar medium. In order to screen Laccase producing strains nutrient agar medium containing 0.5mM Guaiacol was used. 16 strains of *Bacillus* were identified by biochemical methods and one of them with high laccase production selected after enzyme assay. For phylogenetic identification, Bacterial DNA was extracted and the 16S rDNA was amplified using universal 16S rDNA PCR primers. The product of PCR was determined on 1% agarose gel and was observed 1500bp band.

Result: According to morphological and biochemical characteristic, isolated bacteria had similarity to *Bacillus*. Therefore phylogenetic relationship of sequence of isolated bacteria with related strains sequence was considered in genebank using Blast. The comparative analysis of 16S rDNA sequence revealed that it was close to the members of *B. subtilis*.

Conclusion: The presence of laccase has been confirmed on CotA, which is the endospore coat component of *Bacillus* genus, so that it can be expected production of laccase by our isolated bacteria. To our knowledge this is the first report of isolation of this strain from wastewater of a pulp and paper industry.

Keywords: Laccase; *Bacillus subtilis*; Phylogenetic Identification