Abstract

Present study was focused on the ability of thermophilic bacterial strain *Anoxybacillus rupiensis* to detoxify industrial waste containing azo dyes or textile fabric dyes. The strain was optimized and was confirmed phylogenetically and morphologically. Methyl violet, textile industrial waste from one of the textile mills of Faisalabad, market fabric dyes and dyed fabric cloth were decolourized with the help of laccase enzyme secreted by *A.rupiensis*. Decolourization was solely due to enzyme and not due to physical adsorption of dyes on microbial cells surfaces because on centrifugation of dye decolourizing bacterial culture the obtained pellet was totally colorless. Maximum decolourization of 62% was observed at 60°C when dye solution (filtered effluent) inoculated with bacterial culture was incubated for 5 days. Inducers such as guaiacol and $CuSO_4$ were added in media containing bacterial cultures to enhance laccase production. Laccase presence was confirmed by using syringaldazine as a substrate and development of purplish color was observed. PCR amplification of laccase gene was carried out and gene of interest was inserted into cloning vector (pTZ57R/T) and transformed into *E.coli* DH5α. Finally cloned gene was expressed by transformation of recombinant expression vector (pET-21 containing laccase gene) in *E. coli* BL21. Expression of un-induced and IPTG-induced laccase was observed on SDS-PAGE. The protein band of laccase was found to be of 55 kDa.