Cloning and expression of a cellulase gene from newly isolated *Bacillus subtilis* D1

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D1 microbial strain, which has high cellulase activity, was characterized and identified as *Bacillus subtilis* by analysis of 16S rDNA sequence and biochemical studies. The cellulase gene was cloned from *B. subtilis* D1 genomic DNA by polymerase chain reaction (PCR). The amplified PCR product was ligated with the T&A cloning vector and the constructed plasmids were transformed into *E. coli* DH5α. The sequence analysis of the insert DNAs revealed the identification of a 1,499-bp region containing cellulase open reading frame. According to cellulase gene sequence analysis, *B. subtilis* D1 had gene sequence similarity of 98% with *B. subtilis* strain AH18 cellulase gene (EF070194.1). The recombinant plasmid which was ligated with pET-28a(+) vector was expressed in *E. coli* BL21 and the expressed fusion protein was analyzed by SDS-PAGE. A new specific band with molecular weight of about 50 kDa was obtained from cellular extract of *E. coli* BL21 harboring cellulase gene. IPTG concentration and induction time were optimized for expression of cellulase gene. Optimal IPTG concentration and induction time 0.1 mM and 1 hr, respectively.