



ABSTRACT

Bacillus subtilis NRRL-B 941 taken from the culture stock of Institute of industrial biotechnology, GCU Lahore, was used for the study of fermentation process optimization, recovery and characterization of a biopolymer, poly(β -hydroxybutyrate). The selection of this bacterium is related to some of its unique properties such as self-lysing genes expression just after the completion of PHB biosynthetic process which aids in timely and easy recovery of biopolymer. Lack of toxic LPS in *Bacillus subtilis* provides a safe source for the production of biodegradable PHB having properties comparable to those of conventional plastics and can be used in replacement of synthetic plastics. The optimization of fermentation process resulted in the maximum PHB yield with glucose (2%) as substrate and $(\text{NH}_4)_2\text{SO}_4$ (1.5g/L) as nitrogen source. The optimized physical parameters were found to be pH 7, 37°C and 200 rpm agitation speed. The highest rate of PHB accumulation was observed in 24 h old inoculum at 3% (v/v) concentration. *Bacillus subtilis* NRRL-B 941 accumulated maximum PHB (5.78 ± 0.2 g/L) at 72 h of incubation period to be declared as the time for bacterial stationary phase. Various recovery/ extraction techniques were also conducted to determine the proficient one. The final confirmation of PHB through its characteristic functional groups was done by FTIR. The IR spectrum of the biopolymer (PHB) confirmed the presence of CH_3 , CH_2 , $\text{C}=\text{O}$, $\text{C}-\text{O}-\text{C}$ marker functional groups of scl PHAs. Further, thermal profile of PHB was determined by DSC, according to which T_g , T_m , and T_c values were calculated as 3.6°C, 176°C and 50°C, respectively. The TGA results showed the better thermal stability of PHB (T_o : 258°C, T_p : 282°C and T_f : 300°C) as well as less % crystallinity (X_c) of 41 % as compared to that reported in literature. *Bacillus subtilis* NRRL-B 941 being nontoxic and a good producer of PHB that can efficiently replace synthetic plastics is able to compete as a potential candidate for commercial production of PHB. Moreover, this is the first report regarding PHB production from *Bacillus subtilis* NRRL-B 941 strain.