ABSTRACT

The present study deals with the production of cell-free hyaluronic acid by using a mutant strain of *Bacillus subtilis*. Bacterial strain was improved by chemical method and different cultural parameters were optimized. HA production was aseptically carried out by using submerged fermentation. Strain improvement was done by induced chemical mutagenesis of wild-type *B. subtilis* IIB-9 by using HNO_2. Different HNO_2 concentrations (2-12 mM) and different exposure time (5-30 min) were investigated. The final mutant derivative (NA-cys3) was able to produce 5.24±0.26 µg/ml of HA which is highly significant (*HS*, $p \leq 0.05$). The selected wild-type and mutant strain were optimized for various parameters viz. medium (M4), initial pH (7), inoculum size (8%) and incubation time (48 h for mutant and 72 h for wild-type) in batch fermentation. Kinetic studies viz. time course comparison of specific growth rate, yield coefficients, volumetric rates and specific rate constants were also performed. Overall comparison of kinetic parameters for enhanced HA production in batch culture was significant. Repeated batch fermentations (up to 3 consecutive cycles) for enhanced HA production were performed in batch culture using 500 ml Erlenmeyer flasks. The mutant gave 1.92-fold higher HA production than wild-type in batch-I. However, batch-II and batch-III were also found to be encouraging ($p \leq 0.05$). Effect of different sizes of non-woven fabric (NWF 0.1-1 g) on HA production was also observed. The study of HA production at different temperatures (23-65°C) shown that 37°C is an optimized temperature for higher production. It was concluded that the mutant strain exhibited higher HA production, thus could be commercially an attraction for biotech-based industries.