

ABSTRACT

Penicillinase deficient strains of *E. coli* and *B. megaterium* were isolated from the local habitat for the production of intra-cellular and extra-cellular enzyme penicillin-G-Acylase (PGA), respectively. After screening, the best enzyme producing strain of *E. coli* (PCSIR-102) and *B. megaterium* (5-B) were mutagenized by UV, MNNG, SDS and EMS reagents. The strain of *E. coli* (MNNG-37) gave higher enzyme PGA activity (231 IU mg⁻¹) in fermentation medium of composition: Peptone 2%, KH₂PO₄ 0.3%, K₂HPO₄ 0.7%, MgSO₄.7H₂O 0.02%, sodium-L-glutamate 0.5% and yeast extract 0.5%. at pH 6.0, 30 °C temperature for 50 hours. The strain of *B. megaterium* (MNNG-9) gave more higher enzyme PGA activity (329 IU mg⁻¹) in fermentation medium of composition (g/l): yeast extract 2.0; peptone 5.0; sodium chloride 5.0 (Oxoid) at pH 7.0, temperature 37 °C for 45 hours.

Strain of *B. megaterium* (MNNG-9) after treatment with EMS was further mutagenized with in MNNG (Two-stage mutagenization) which gave higher enzyme activity of strain M-9 (525 IU mg⁻¹) in molasses medium of composition (g l⁻¹): CaCl₂.2H₂O 0.05; K₂HPO₄ 1; MgSO₄.7H₂O 0.5; phenyl acetic acid 10; molasses 12 and Soytone 30 at pH 7.0, temperature 37 °C for 30 hours.

The specific activity of PGA by *E. coli* (PCSIR-102) and *B. megaterium* (5-B) was also studied by using different substrates like wheat bran, rice hulls and defatted oil seed cakes of soybean, sunflower or cotton by solid substrate fermentation. Low activity of PGA was found as compared to the submerged fermentation.

The cells of *B. megaterium* (M-9) were immobilized in calcium alginate beads. The specific activity of PGA was (505 IU mg⁻¹) after 15 hours of fermentation at 30 °C. However, the enzyme PGA produced from *B. megaterium* (M-9) was immobilized giving better enzyme activity as compared to the immobilized whole cells and free cells.

88% of the enzyme PGA from *B. megaterium* (M-9) was recovered from the fermentation broth. The level of purification was also confirmed with the help of Fast Performance Liquid Chromatography