ABSTRACT

The present study deals with the enhanced production of enzyme cutinase from fungus *Aspergillus oryzae* ISL-9 by optimizing growth parameters and use of strain improvement strategy. The strain was obtained from Institute of Industrial Biotechnology, GCU Lahore. The chemical mutagenesis was induced by using methane methyl-sulfonate (MMS). The effect of different MMS concentrations (0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mM) and different exposure time (5-30 min) was investigated. Resistance against diversion was developed by using 30 ppm of L-cysteine HCl. The final mutant derivative MMS-L-cys-3 was able to produce 38.09±0.90 mU/ml of cutinase which was significantly higher than the wild-type 19.13±0.98 mU/ml. The wild-type and the potent mutant strain were examined under SEM. The differences in terms of sporangiophore, arrangement of the phialides and total number of conidiospores were observed. Submerged type fermentation was carried out to produce the enzyme while using apple cutin as a substrate. The wild-type (ISL-9) and mutant strains (MMS-t8, MMS-L-cys-3) were optimized for various parameters viz. substrate level (3%), medium pH (6.5) and time of incubation (48 h). The cutinase activity was further stimulated by the inclusion of various micro-minerals and stabilizers. The most notable finding was the addition of 25mM $CaCO_3$, which increased the enzyme activity significantly. After the optimization of parameters for enzyme activity, the potent mutant showed a 1.4-fold increase in the enzyme activity as compared to the wild-type. The polyester hydrolysis potential of the cutinase produced by the wild-type and mutant strains was also evaluated. Under the optimized conditions of 60% enzyme concentration, 5 h of time of incubation and 50°C temperature, an overall of 41% of raw polyester was hydrolyzed by the potent mutant strain MMS-L-cys-3, which was significantly higher than the rate of polyester degradation by the wild-type ($p \leq 0.05$).