ABSTRACT

The present study deals with the enhanced productivity of L-asparaginase from the fungus *Aspergillus oryzae* by optimization of parameters and use of strain improvement strategy. Thirtyfive different fungal strains were isolated from different soil samples. After screening, ISL-3 (*A. oryzae*) and ISL-9 (*A. niger*) proved to be better isolates. Solid state fermentation was carried out using sugarcane bagasse as a substrate. The selected isolates were optimized for various parameters viz. substrate level of 5 g, MC-5 (9 ml) as a diluent, and incubation time 72 h. After optimization, ISL-3 (*A. oryzae*) exhibited 12.15% better yield as compared to ISL-9 (*A. niger*), therefore, former was selected for further investigation. Strain improvement was done by induced chemical mutagenesis of wild-type *A. oryzae* ISL-3 using nitrous acid. Different NA concentrations (3, 6, 9, 12, 15 and 18 mM) and different exposure time (5-30 min) were investigated. The final mutant derivative (NA-t3) was able to produce 4.479±0.22 U/g of LA which is highly significant (*HS*, $p \leq 0.05$). Resistance development in NA-cysL-C4 was performed using 8 ppm of L-cysteine HCl. Electron microscopy revealed morphogical characteristics of wild- type and mutant strain. The LA activity was further stimulated by inclusion of various additive viz., $MgSO_4.7H_2O$ (3 mM), ammonium nitrate (0.2%) and 2-ME (0.125%). The most notable finding was with addition of stabilizers which enhanced the enzyme activity significantly. The final mutant cell yielded 16.122±0.81 U/g of LA which is 2.07-fold higher than the wild-type. The enzyme was partially purified with 35-85% ammonium sulphate precipitation followed by dialysis and chromatography. The yield of mutant strain after purification was found to be 47%. The molecular weight of the enzyme from wild-type and mutant strain was found to be 120 kDa on SDS-PAGE. It was concluded that the mutant strain exhibited higher LA yield and thus, could be commercially an attraction for the enzyme producing community.