ABSTRACT

The present work discussed the production and characterization of an extracellular acetyl xylan esterase (AXE) from a thermophillic *Alkalibacillus* spp. Twenty different strains were isolated from soil samples of three different habitat i.e. industrial, agricultural and mountainous range of salt affected area. After primary and secondary screening Isl-3 was selected as the highest producer (5.25±0.52 IU/g) of AXE. The wild type of Isl-3 was mutated to improve the production of AXE by different conc. of EMS, ranging from 0.25-1.5 mM and the better activity (18.95±0.95 IU/g) was recorded at 0.5 mM. The time exposure for the same mutagen was varied from 5-30 min and a mutant coded, EMS-t3 was selected as it provided maximum enzyme activity of (19.47±0.97 IU/g) at 15 min of exposure. The resistance was developed by addition of L-cysteine HCl to protect it from back mutation and environmental conditions or through its natural repair mechanism. To enhance the production of AXE different physical and nutritional parameters were optimized and the enzyme activity of wild strain was compared with the mutant variant. The substrate level was varied from 2.5-15 g and maximum activity of 6.17±0.61 IU/g for wild type whereas 21.15±1.05 IU/g for mutant variant was obtained at the level of 7.5 g. A remarkable increase in enzyme activity was detected when the physical parameters including initial moisture content (100 ml), time of incubation (48 h), initial pH (9.5) and temperature (45°C) were further optimized. The maximum production of AXE was observed (36.47±1.82 IU/g) with the addition of 0.3% Tween 80 as an organic nitrogen source, while addition of 0.2% ammonium nitrate as an inorganic nitrogen source provided the activity of 39.14±1.95 IU/g. The size and age of inoculum was optimized to 1 ml and 48 h, respectively. The role of different inhibitors were also noted and $COCl_2$ was optimized and the enzyme activity was detected as 46.15±2.30 IU/g. From the results it is clear that there is an overall increase of approx. 8.7 fold in net enzyme activity of mutant variant as compared to wild type.