



## ABSTRACT

The present study deals with the production and characterization of an extracellular laccase from a thermophilic *Alkalibacillus* spp. Sixteen different strains of thermophilic *Alkalibacillus* spp. were isolated from their natural habitats, including tap water, pond water and fresh water. The isolate (Isl-5) exhibiting better enzyme activity ( $6.55 \pm 0.655$  IU/g) was selected after primary screening. The selected isolate, Isl-5 was mutated to improve the production of enzyme by using methyl methane sulfonate (MMS) as a chemical mutagen. The concentrations of MMS were ranged from 0.25-1.5 mM and the better enzyme activity ( $12.93 \pm 0.64$  IU/g) was observed at 0.5 mM concentration. The exposure time for the mutagen was changed from 5-35 min and a mutant coded, MMS-t6 was selected as it provided maximum enzyme activity of ( $13.2 \pm 0.66$  IU/g) when exposed for 25 min. The selected mutant variant was made resistant against L-cysteine HCl, to prevent it to back mutate either due to environmental conditions or through its natural repair mechanism. Both the wild-type and putative mutant were compared in terms of their enzyme activity. The production parameters were then optimized to enhance the production of enzyme. The substrate level was changed from 5-30 g and maximum activity ( $26.89 \pm 1.34$  IU/g) was noticed by the mutant variant, when 20 g substrate was supplied while the activity of the wild remained to ( $12.06 \pm 0.60$  IU/g) only. The moisture level, pH and temperature were also optimized to be as 15 ml, 9.5 and  $60^\circ\text{C}$ , respectively. The optimization of nutritional conditions resulted in maximum activity ( $84.62 \pm 4.23$  IU/g) when  $\text{NaNO}_3$  was used as an inorganic nitrogen source while addition of Tween 80 as an organic nitrogen source exhibited better activity ( $88.62 \pm 4.43$  IU/g). The size and age of inoculum was optimized to be 1 ml and 48 h, respectively. The effect of various inhibitors was also examined, where EDTA inhibition was recorded to be significant and provided very low enzyme activity ( $36.08 \pm 1.8$  IU/g). Overall, there was a 16 fold increase in the activity by the mutant variable as compared to the wild-type which is highly encouraging ( $p \leq 0.05$ ).