ABSTRACT

Alpha amylases comprise around 25-30% of the world's enzyme utilization. They are widely used in various industries. The naturally occurring alpha amylases from *Bacillus* sp do not have great potential to use in harsh industrial conditions. Alpha amylases used on industrial scale must have great attributes like high activity and stability at adverse temperature and pH, high productivity and economical. To achieve this target, the present study was conducted to improve the wild type strain of *Bacillus subtilis* through UV mutagenesis to enhance its enzymatic stability and activity. The wild type strain was subjected to UV irradiation followed by optimal mutation conditions (3-4 min UV exposure time, 20 cm distance, 254nm wavelength, and 20W power). Total 1100 mutants were screened on the basis of zone of hydrolysis. Finally, three mutants (HM-1, HM-2, and HM-3) were selected qualitatively. Among these three mutants, HM-2 gave up to 4-fold (4 times) increased alpha-amylase activity at optimal temperature 60°C, and pH 7.0 compared to other mutants and wild type (WT-1). The alpha amylase from this mutant strain (HM-2) was also highly thermostable, alkaline, and thermophilic as it showed stability in the pH ranges 5.0-8.0 and temperature ranges 40°C-80°C. The amylase activity got stimulated in the presence of high concentration (5mM) of $MgSO_4$ and $CaCl_2$ but inhibition was observed in the presence of $HgCl_2$ and $CuSO_4$ at the same concentration. Similarly, SDS and Tween-20 inhibited the enzyme activity at high concentration of 5mM than 2mM. But EDTA and Triton X-100 stimulated the enzyme activity at 5mM and inhibited at low concentration of 2mM. Thus, this α-amylase would be a potential candidate in various industries.