

# ABSTRACT

The present work describes the isolation, screening and selection of bacterial cultures belonging to the genus *Bacillus* for the production of alkaline protease. One hundred and five *Bacillus* cultures were isolated from soil samples collected from different tannery areas in sterile polythene bags. The strains were selectively isolated on the basis of larger relative haloes (H/C ratios) on peptone-yeast extract-casein agar plates. The selected cultures were transferred to nutrient agar slants and were stored in a refrigerator at 4°C for maintenance and screening. The quantitative screening for alkaline protease production by the isolated strains was carried out in shake flasks and the most potent strain, IH-72 producing  $4.79 \pm 0.03$  U/ml of enzyme was selected. The strain was identified on the basis of standard morphological and biochemical tests as *Bacillus subtilis* and was assigned the code IH-72.

The selected culture was subjected to mutagenic treatments with physical (UV and gamma radiations) and chemical (MNNG, EMS and nitrous acid) mutagens to improve its proteolytic potential. Several mutants with enhanced protease production were isolated after treatments with one or more mutagens, but the mutant EMS-8 was found to be the best showing highest production ( $9.53 \pm 0.03$  U/ml) of alkaline protease and was given the code as *Bacillus subtilis* IH-72<sup>EMS-8</sup>. This mutant showed an overall increase of about 100 % over its parent strain for the production of alkaline protease.

The fermentation experiments for the production of alkaline protease by both the wild and mutant strains of *Bacillus subtilis* IH-72 were carried out in 250 ml Erlenmeyer flasks and a laboratory scale 7.5 L stirred fermentor. The production of alkaline protease was enhanced by optimization of cultural conditions for both the wild and mutant strain.