Abstract

Bacteria are widely known for their potential to produce and excrete extracellular enzymes into the environment. *Bacillus subtilis* has the ability to produce a wide variety of industrially important enzymes. *Bacillus subtilis* was isolated from the agriculture soil and identified by cultural, morphological and biochemical characteristics. Finally, by Ribotyping it was confirmed that the isolated bacterium was *Bacillus subtilis*. Fermentation media was optimized to induce *B. subtilis* to produce protease enzyme. Proteases are the group of enzymes that hydrolyze the peptide bonds of proteins to liberate amino acids. Fermentation media was optimized for carbon and nitrogen sources and also for pH and temperature. The best carbon source was dextrose and the best nitrogen source was peptone. The optimum temperature and pH for the production of protease were 37˚C and 8.0, respectively. Hence, fermentation for the production of protease was carried out with optimized carbon and nitrogen sources and at 37˚C and pH 8 for 48 hours in a shaking incubator (150 rpm). Protease product was harvested and partially purified by ammonium sulphate precipitation. Protease was characterized, optimum temperature for the activity of protease was 37˚C and it was stable at 37˚C for 1 hour and the stability started to decrease at higher temperature. Optimum pH for the activity of protease was 8.6 (Tris-HCL buffer) and it was stable at 8.6 pH for 1 hour and the stability started to decrease at the pH higher than 8.6. Hence, this protease was characterized as mesophilic alkaline protease. Protease was stimulated by metal ions like Mg^{+2}, Ca^{+2} and Cu^{+2} while other metals such as Zn^{+2}, Na^{+} and EDTA inhibited the enzyme activity. Effect of different inhibitors on protease activity was also determined and different inhibitors like EDTA, SDS and triton inhibited the protease activity most effectively at the concentration of 30mM. Of all the detergents studied, triton (30mM) was the most effective to considerably inhibit (50%) the protease activity. Hence, isolated protease enzyme has biotechnological potential and can be exploited commercially.