Abstract

Thermostable proteases are important industrial and commercial enzymes. The present study is centered on the isolation of thermophilic bacterial isolate to produce thermostable protease. The isolated strain of bacteria was identified as *Bacillus smithii*. Static fermentation was employed with a primary focus on the production and optimization of various fermentation parameters to achieve maximum thermostable protease activity. Different substrates, i.e., soyabean meal, wheat bran, lyophilized whey, and rice bran were assessed, with the highest thermostable protease production with soyabean meal. A range of incubation periods (12, 24, 48, and 96 hrs) were checked for thermostable protease production and highest production was found after 48 hrs of incubation. Different incubation temperatures (50, 55, 60, 65, 70, and 75℃) were analysed and highest production of thermostable protease was observed at 55℃. Fermentation media were incubated with different values of pH, i.e., 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, and maximum thermostable protease production was achieved at pH 7.5. Different sources of carbon (glucose, starch, sucrose, fructose, lactose, galactose, and maltose) were tested and the highest production of thermostable protease was assessed with glucose. Various organic nitrogen sources, such as, peptone, yeast extract, urea, and casein, were added in the fermentation medium and highest protease production was observed with peptone. Whereas, different inorganic nitrogen sources, i.e., ammonium nitrate, ammonium citrate, ammonium chloride, and ammonium sulphate, were also assessed and maximum protease production was found with ammonium nitrate. To further enhance the thermostable protease production, different metal ions (Mg^{2+}, Cu^{2+}, Ca^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, K^{1+}, Na^{1+}) and different inoculum concentrations (0.5-5%) were added in the fermentation medium. The maximum thermostable protease production was recorded with Mg^{2+} and 3% inoculum concentration, reporting the highest units, i.e., 25.9 U/ml. The results of this study provide valuable insights into optimizing thermostable protease production with implication for sustainable and eco-friendly applications.