ABSTRACT

Owing to immense physiological, biotechnological, biomedical as well as commercial importance, currently fungal proteolytic enzymes draw the attention of researchers in many ways. While studying production of some glycosidases from the fungus *Termitomyces clypeatus* MTCC-5091 mainly sucase, proteolytic activities were detected in the culture medium. These proteases were not studied earlier. Therefore the thesis work was undertaken with an aim to study the regulation of the protease enzyme in the fungus. The fungus produced two proteases, acidic metalloprotease (AcP) and alkaline serine protease (AkP). Secretion of AcP was constitutive while AkP production was inducible and substantially dependent on external protein supplements into media. Both these proteases were monomeric and non-glycoprotein in nature. Though biochemical, kinetic properties and application potencies were appreciably different for AcP and AkP. Both the enzymes were purified to homogeneity and characterized biochemically. AcP showed high milk-clotting potency that preferentially hydrolyzed the peptide bond in Phe_{105}-Met_{106} of k-casein, analyzed by Urea-PAGE and LC-ESI-MS. Purified AcP (29 kDa) had pI value 4.6 and optimally active at pH-5 and 45°C. One-and two dimensional zymographies revealed a single polypeptide band with proteolytic signal. The enzyme can be developed as a substitute for chymosin. The thesis works report the production, optimization, purification and applications of non-collagenolytic AkP from *T. clypeatus* focusing on bioremediation and biomedical area. AkP titre was successfully optimized through the Plackett-Burman and RSM using the Box-Behnken design. As an ecofriendly alternative, AkP showed significant promise for bioremediation and industrial applications through time-saving bioprocesses in reduction of BOD and COD values of direct CETP inlet of tannery wastes, goat hide dehairing and bird feather detachment. The sequence of first 15 N-terminal amino acids of AkP (33 kDa) showed high homology with other serine proteases. AkP had pI value 8.9 with optimal activity achieved at pH-10 and 45°C. Studies were also performed for investigating *in vitro* antiproliferative potency of AkP on human HepG2 cancer cells. AkP treatment resulted in G2/M arrest and apoptosis in HepG2 cells. Hence, the edible mushroom *Termitomyces clypeatus* is a new source of two important proteases that can be explored in future for wide-ranging application potential.