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Serine protease from *Bacillus* sp. WRD-2: gene cloning, expression, purification and enzymatic properties

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A gene, *isp-Y*, encoding an intracellular serine protease from a newly isolated *Bacillus* sp. WRD-2 was cloned and characterized. Nucleotide sequence analysis showed an open reading frame of 960 bp which could encode a polypeptide comprised of 319 amino acids. The primary structure of this enzyme predicted the structural features characteristic of other intracellular serine proteases including active sites, Ser, His and Asp, as well as no signal sequence. The predicted amino acid sequence showed more than 60% homology with those of intracellular serine proteases from *Bacillus* species. When expressed in *E. coli*, the recombinant enzyme (rIsp-Y) was overproduced in the cytoplasm as soluble and active form. The purified enzyme was completely inhibited by phenylmethylsulfonyl fluoride, EDTA and antipain. The enzyme had maximum activity at pH 8.0 and 45℃. It was stable at pHs from 7.5 to 11.0 and below 50 ℃.