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Purification and Characterization of extracellular serine protease produced by *Sarcodon aspratus*

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Extracellular protease from *Sarcodon aspratus* was purified and characterized. The molecular mass of the protein was determined to be 30kDa by SDS-PAGE. When the protease was treatead with various inhibitors and divalent cations, protease activity was completely inhibited by 1mM PMSF specific for serine proteases and 5mM Cu^{++}. Synthetic substrate N-Succinyl-Ala-Ala-Pro-Leu-pNA could be used for the fluorimetric assay of the protease. The protein was found to have carbohydrate residues as its component which was estimated to be 12.8% of protein. The enzyme had maximum activity at pH9 and pH11 using casein and synthetic substrate as a substrate, respectively and stable relatively at a basic condition. Optimal temperature of the protease activity was at 65℃ and completly inhibited when the protein held at 70℃ for 30min. Both activity and concentration of the protease drooped by 50% after incubated at 62℃ for 30min. This suggested that autodigestion occured. Gradual redution of band intensity was observed as time proceeded at 70℃ on the SDS-PAGE.

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Screening for mammalian Topoisomerase I Inhibitors from Actinomycete strains

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DNA topoisomerase I (Topo I) is one of major targets for screening of anticancer agents. Topo I from calf thymus was previousely used for our screening work. To screen with more proper mammalian enzyme, Topo I from human carcinoma cell line (HeLa S3) was used for bioassay. Hela S3 cells were grown in DMEM supplemented with 10% horse serum. The nuclei protein was extracted with buffer solution containing 2M NaCl, 50mM Tris-HCl(pH7.5), 1mM PMSF, and 10mM 2-mercaptoethanol. The DNA was precipitated by slow addition of polyethyleneglycol 6000 with final concentration of 2%. The nuclei protein supernatant was further purified with BioRex 70 and hydroxylapatite column. Isolated strains showing cytotoxic activity against p388 and DLD-1 cell line were selected. The inhibitory activity against topoisomers I was evaluated by relaxation assay on agarose gel. Strain 8301 isolated from Antarctic soil, strain 8202 from sea sand and strain 8044 from soil of Temperate Zone inhibited topoisomerse I activity of HeLa cell.