# Purification and some Biochemical and Immunological Characterization of a Protease from Serratia Marcescens.

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## Abstract:

An extra cellular protease purified 30-fold was induced in cultures of Serratia marcescens (NCIB 1377) during growth in liquid synthetic medium containing vitamin-free casein (sigma) as the inducer. Purification was by means of ammonium sulphate precipitation and chromatography on sephadex G-100 and DEAE-sephadex (A-50) columns. The molecular weight estimated by gel filtration was approximately 45,000. Optimum temperature and pH of activity, using casein as substrate were $40^{o}$c and 8.5 respectively. The protease was stable for 60 minutes at 30-$40^{o}$C, losing all detectable activity at $60^{o}$C, even for 10 minutes.

$Ca^{++}$ and $Mg^{++}$ did not affect the enzyme activity. Sulfhydryl reagents, IAA, dithiothreitol and L-cysteine could not inhibit its activity; and metal chelators, dithizone and NacN failed to be inhibitory to the protease activity. However, EDTA, at relatively high concentrations inhibited the protease activity. Inhibition of enzyme activity by 2,4-Dinitrophenol indicated need for metabolic energy in enzyme activity.

The protease was well inhibited by PMSF indicating it is a serine enzyme. The protease digested a wide range of proteins but with a preference for the milk proteins. It possessed an apparent km of approximately 0.75mg/ml for casein.

The sephadex G-100 fraction of the protease was used to raise antibodies in locally bred rabbit. The antibody to this protease was found to inhibit its enzymic activity. Ouchterlony double-diffusion tests revealed antigenic relatedness between all the enzyme fractions at the different stages of purification.

The protease shares no antigenic relatedness with trypsin, chymotrypsin, papain and carxypeptidase enzymes.

**Keywords:** Metabolism/ enzyme/ protease/ gel filtration/ precipitation/ chromatography/ Ouchterlony double-diffusion tests/ antigens

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