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Identification and Characterization of Proteolytic Enzymes secreted by *Toxocara canis* infective larvae with special emphasis on a 50 kDa protease

By

Y.L.P.Jayananda
B.Sc (Hon) Zoology

Department of Pathobiology
Faculty of Veterinary Medicine & Animal Science
University of Peradeniya

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Thesis Supervisors: Prof. R.P.V.J. Rajapakse B.VSc (SLan), PhD (Perad)

Dr. S.B.P. Athauda B.Sc (Perad), PhD (Tokyo)

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Abstract

Toxocariasis is a parasitic disease caused by the accidental ingestion of eggs of parasitic nematode *Toxocara canis*. Proteolytic enzymes secreted by the larval stages of *T. canis* are suggested to as important biological molecules in their tissue migration process. Therefore, an attempt has been taken to identify a larval protease which might be important in tissue migration, thereby, targeting the particular enzymes against *Toxocara* infections.

Excretory-Secretory Products (ESP), collected from *in vitro* cultures of infective larvae of *T. canis* were examined for the presence of proteolytic enzymes by gelatin-zymography and were characterized according to pH optima, substrate specificity and inhibitor susceptibility. A 50 kDa protease was partially purified by using DEAE – anion exchange chromatography and its activity was characterized for optimum pH, temperature and inhibitor sensitivity.

In the preliminary identification of protein fractions in ESP of *T. canis* larvae, SDS-PAGE revealed minimum of 11 protein fractions. Out of the 11 bands, 7 were showing the proteolytic activity in zymography and their molecular weights were ranging between 20 kDa to 175 kDa. The optimal pH value for these protease activities was observed between 5.5 to 7.0. Activity was optimum against albumin over gelatin and casein. Serine, cysteine and metalloprotease were found to predominate. A 50 kDa protease was partially purified by using DEAE- anion

exchange chromatography and its activity was inhibited by metalloprotease inhibitor EDTA (10 mM). Its optimum activity was detected in pH 8.5 at 70° C.

T. canis ESP proteases demonstrate heterogeneity based on their differential migration in polyacrylamide gels containing gelatin. Hence, these proteases might have functional variations during larval migration. The 50 kDa enzyme might play a specific function in the course of migration. Inhibition of its activity might result in the collapsing of larval migration, thus can be instrumental in arresting toxocariasis.

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