CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2014 Vol. 3 (4) October-December, pp.59-62/Arunkumar et al.
Research Article

ASSESSMENT OF MATRIX METALLOPROTEINASE (MMP) ACTIVITY IN EXCRETORY/SECRETORY ANTIGENS OF *GASTROTHYLAX CRUMENIFER* FROM BUFFALO BY GELATIN ZYMOGRAPHY

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ABSTRACT
In the present investigation, matrix metalloproteinase (MMP) activity was assessed in Excretory/Secretory antigens of *Gastrothylax crumenifer* from buffaloes by gelatin zymography. Live adult flukes were collected from local abattoirs and washed thoroughly with Phosphate buffer saline (pH 7.4). The adult flukes were suspended in DPBS (pH 7.2) and incubated at 37°C in a BOD incubator for 8 hours. The material was centrifuged at 7000 rpm for 30 min at 4°C and the supernatant collected was designated as E/S antigens. Through gelatin zymography, it was observed that the presence of prominent bands at 220 kDa, 92 kDa, 72 kDa in E/S antigens of *Gastrothylax crumenifer*. The 135 kDa size was observed as a fainter band. Among the four bands, 92 kDa band was showing the greatest gelatinolytic activity. The gelatinase activity of MMP- 9 was purely from E/S antigens of *Gastrothylax crumenifer* as it was thoroughly subjected to enough prewashing treatments to assure the MMP activity was solely from *Gastrothylax crumenifer* alone. The relative amount of 92 kDa band and to that of 72 kDa was 1.5: 1. The ratio of MMP 9/2 was observed as 1.5 in E/S antigens of *Gastrothylax crumenifer* suggesting the activator role of MMP-2 in regulating the activity of MMP-9.

Keywords*: Gastrothylax Crumenifer, MMP, Gelatin Zymography, Buffalo*

INTRODUCTION
Matrix Metalloproteinase (MMP) and, in particular, of gelatinases in both helminth and protozoan infections in animals has been documented well earlier. The parasitic helminths are the major group of invertebrates, which dwell in different organs of vertebrate hosts. MMPs can be the potential therapeutic targets to prevent the disruption of several barriers. Many of these parasites to complete their life cycle have to migrate or invade through various organs of their hosts. During this journey, expression and release of MMPs by various parasitic nematodes have been associated with the pathology resulting from histolysis (Lai *et al.*, 2005). According to their substrate specificity, MMPs can be categorized as collagenases, gelatinases, elastases, Stromelysins and membrane-type MMPs. Nematode MMPs generally include collagenases, gelatinases and elastases (McKerrow *et al.*, 1990; Healer *et al.*, 1991). Most of these works evaluated MMPs from excretory/secretory (ES) products as well as the extracts of parasitic nematodes (Lai *et al.*, 2005). But the work on MMPs in parasitic trematodes, particularly in ruminal amphistomes is very limited.
Hence, the present study was undertaken to assess the gelatinase activity in excretory/secretory antigens of *Gastrothylax crumenifer* by gelatin zymography

MATERIALS AND METHODS
Live, mature *Gastrothylax crumenifer* were collected from the rumen of Indian water buffaloes (*Bubalus bubalis)*, slaughtered at the local abattoirs of Pattukkottai and Thanjavur. Worms were thoroughly washed in Phosphate Buffer Saline without glucose, pH 7.4 and pre maintained at 37ºC. After careful preservation in PBS, the worms were immediately transferred to the laboratory for further processing. Excretory-secretory antigens (ES-Ag) were prepared as per Saifullah *et al.*, 2011 with minor modifications. Live

intact adult flukes were weighed and suspended in DPBS (pH 7.2). After incubation at 37°C in a BOD incubator for 8 hrs, the fluid was centrifuged at 7000 rpm for 30 min at 4°C. and the supernatant was designated as E/S antigens. The E/S antigens were further lyophilized in a centrifugal freeze-dryer and then it was reconstituted in DPBS and stored at -20°C till further use. The protein content of the samples was estimated by Lowry method (1951).

Gelatin zymography of excretory/secretory antigens of *G. crumenifer* was carried out as per the method of Heussen and Dowdle (1980) with some modifications and the procedure was as follows. SDS-PAGE was carried out, as described by the method of Laemmli, (1970). The resolving gel (8%) was co-polymerized with 0.3% gelatin solution (final concentration of gelatin in gel was 0.15%) and the electrophoretic run was carried out at 100 V until tracking dye reaches the bottom. Then, renaturation was carried out with renaturation solution (2.5% Triton X -100) for 3 hours on a mechanical shaker with mild agitation. Then, developing was carried out by incubating the gel in developing buffer (10 mM $CaCl_2$, 0.15 M NaCl and 50 mM Tris (pH 7.5)) for 18 hours at 37ºC and then stained with 0.25% Coomassie blue for 2 hours, followed by destaining for 1 hour with destaining solution and then, further destaining was carried out with distilled water. Then, calibration of the gelatin zymogram was carried out with human capillary blood gelatinases, as per the procedure suggested by Makowski and Ramsby (1996). A drop of human capillary blood (15-20µL) was obtained by finger stick puncture and placed in a tarred polypropylene tube. The weight of the blood was determined in an analytical balance and 20 volumes of non-reducing Laemmli buffer was immediately added. The sample was then vortex mixed for 30 seconds and aliquots stored at -20°C.

RESULTS AND DISCUSSION

The presence of MMP in the E/S antigens of *Gastrothylax crumenifer* was assessed by gelatin zymography. In Gelatin zymography, the presence of three prominent MMP-9 bands at 220 kDa, 92 kDa, 72 kDa of MMP-9 were observed in E/S antigens of *Gastrothylax crumenifer*. The 135 kDa of MMP-9 was observed as a fainter band. Among the four bands, 92 kDa band was showing the greatest gelatinolytic activity. The ratio of 92 kDa MMP-9 to that of 72 kDa MMP-2 was 1.5 and the 72 kDa band in both the forms of pro and active forms were observed as two overlapping bands compared to that of human marker. The gelatinolytic activity of 220 kDa, 92 kDa and 72kDa proteinases were at least five times higher than the human marker. The presence of 72 kDa band was measured by using MMP-2 standard collected from human (CalBiochem Co.) as marker. All the three forms of MMP-9 and MMP-2 were catalytically active and found to be TIMP free and reported for the first time in E/S antigens of *Gastrothylax crumenifer*.

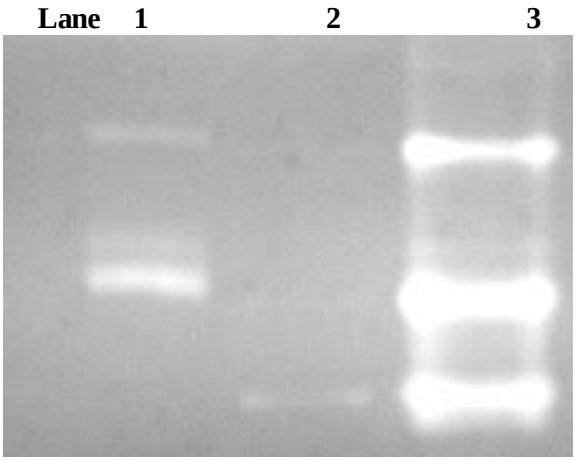

Figure 1: Gelatin zymography analysis of E/S antigens of *Gatrothylax crumnifer*
Lane 1- Human capillary blood markers
Lane 2- Human MMP-2 standard
Lane 3- E/S antigens of *Gatrothylax crumnifer*

The presence of strong gelatinolytic activity in the E/S antigens of *Gastrothylax crumenifer* was authentically confirmed. The presence of MMP-9, MMP-2 was already documented in parasitic infections in the central nervous system (Bruschi and Pinto, 2013). In their study in human serum, the authors reported the presence of both 220 kDa and 95, 82 of MMP-9 and 72 kDa of MMP-2 in patients with neurocysticercosis as well as in healthy controls.

Lai *et al.,* (2005) reported the gelatinase activities in E/S product of *Angiostrongylus cantolensis* and suggested that these MMP secreted by larvae could be associated with parasites spreading and pathogenesis in the host. They further proposed that the MMPs were secreted by the parasite not by the host.

In our study, the gelatinase activity of MMP-9 was purely from E/S antigens of *Gastrothylax crumenifer* as it was thoroughly subjected to enough prewashing treatments to assure the MMP activity was solely from parasite alone not from the host.

MMP-2 is an activator of MMP-9 (Freidman *et al.,* 1995). In our study, the presence of both the pro and active forms of MMP-2 and increased activity of MMP-9 could be related as the MMP-2 was first activated and then the whole pro MMP-9 was activated. The whole pro MMP-9 was activated into its active form. The ratio of MMP 9/2 was observed as 1.5 in E/S antigens of *Gastrothylax crumenifer* suggesting the active role of MMP-2 in regulating the activity of MMP 9. The presence of very low level of MMP-2 to that higher level of MMP 9 is a suggestive of the regulatory role of MMP-2 on MMP-9. The ratio of MMP 9 to MMP 2 was decreased from its normal state to that of diseased conditions.

There are several reports showing varied sources of MMP-2 and 9. Gelatinase B can be synthesized by epithelium lining the parasites. Further gelatinase B was identified as a product of polymorphonuclear leucocytes (Murphy and Dougherty, 1990) and macrophages (Hibbs *et al.,* 1987). As the E/S antigens of *Gastrothylax crumenifer* product was subjected into high speed centrifugation, the possibility of leucocytes liberation of MMP-9 was also eliminated.

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