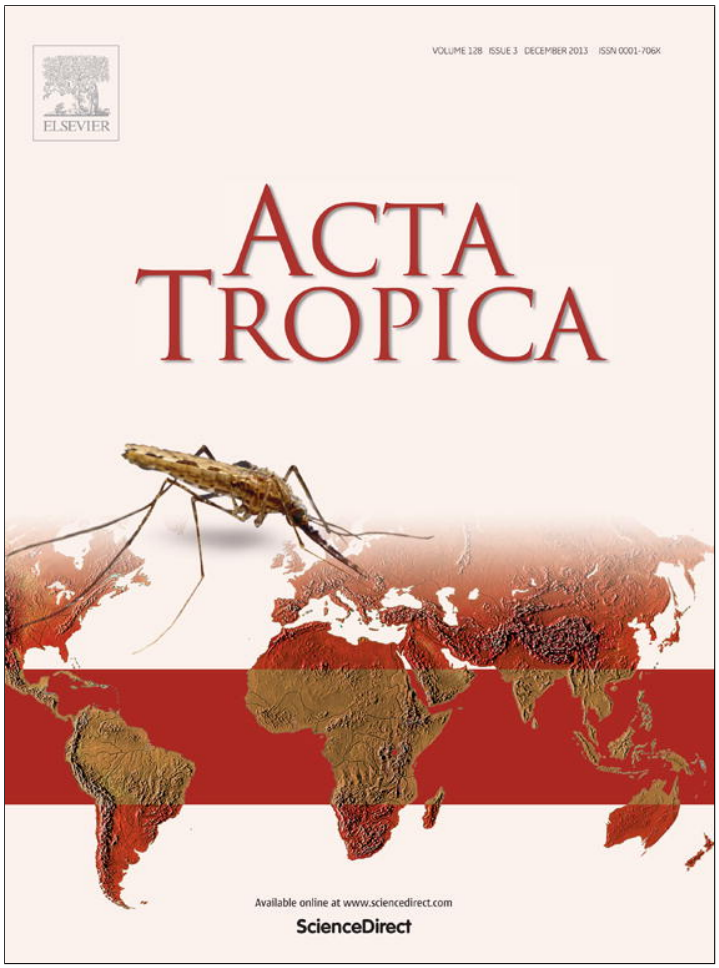

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Proteolytic activity regarding *Sarconesiopsis magellanica* (Diptera: Calliphoridae) larval excretions and secretions

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ABSTRACT

Sarconesiopsis magellanica (Diptera: Calliphoridae) is a medically important necrophagous fly which is used for establishing the post-mortem interval. Diptera maggots release proteolytic enzymes contained in larval excretion and secretion (ES) products playing a key role in digestion. Special interest in proteolytic enzymes has also been aroused regarding understanding their role in wound healing since they degrade necrotic tissue during larval therapy. This study was thus aimed at identifying and characterising *S. magellanica* proteolytic enzyme ES products for the first time. These products were obtained from first-, second- and third-instar larvae taken from a previously-established colony. ES proteins were separated by SDS-PAGE and their proteolytic activity was characterised by zymograms and inhibition assays involving BAPNA (*N*α-benzoyl-DL-Arg-*p*-nitroanilide) and SAPNA substrates, using synthetic inhibitors. The protein profile ranged from ~69 kDa to ~23 kDa; several of them coincided with the *Lucilia sericata* ES protein profile. Serine-protease hydrolysis activity (measured by zymogram) was confirmed when a ~25 kDa band disappeared upon ES incubation with PMSF inhibitor at pH 7.8. Analysis of larval ES proteolytic activity on BAPNA and SAPNA substrates (determined by using TLCK and TPCK specific inhibitors) suggested a greater amount of trypsin-like protease. These results support the need for further experiments aimed at validating *S. magellanica* use in larval therapy.

1. Introduction

Sarconesiopsis magellanica is a diptera from the Calliphoridae family; it is distributed throughout several South-American countries: Argentina (Mariluis and Mulieri, 2003), Bolivia, Chile, Ecuador, Perú (Pape et al., 2004) and Colombia. It is found in Colombia in departments lying 1200 to 3100 meters above sea-level (masl), such as Norte de Santander, Antioquia, Boyacá, Cundinamarca and Bogotá (Martinez et al., 2007; Pape et al., 2004; Pinilla et al., 2012; Segura et al., 2009).

S. magellanica feeds mainly on decomposing material and has been used in a forensic setting for establishing the post-mortem interval (PMI) since it is one of the first colonisers of decomposing bodies, mainly in rural areas (Martinez et al., 2007; Segura et al., 2009) and as it is a hemisynanthropic species (Pinilla et al., 2012). This blowfly has also been shown to have medical and veterinary importance (Amat, 2009; Baumgartner and Greenberg, 1984).

The larvae of *Lucilia sericata*, a species belonging to the same family as *S. magellanica*, are usually used in healing necrotic wounds where they perform functions such as removing necrotic tissue (debridement), have antibacterial properties and stimulate granulation tissue formation (Graninger et al., 2002; Sherman, 2003; Sherman and Shimoda, 2004; Telford et al., 2011). It has been shown that such properties are due to the activity of enzymes contained in larval excretion and secretion (ES) products released by the larvae within a wound (Horobin et al., 2003). Studies in this species have led to identifying and characterising proteolytic enzymes derived from their ES and evaluating *in vitro* effects on fibroblast cultures for a better understanding of how necrotic wounds heal (Chambers et al., 2003; Horobin et al., 2005, 2006).

According to the proteolytic enzymes described in insects so far, these molecules include a large complex of proteases whose most dominant family consists of serine proteases which have been reported as being the insects' main class of digestive enzymes (Casu et al., 1994; Johnston et al., 1995; Kerlin and Hughes, 1992; Sandeman et al., 1990; Young et al., 1996). This family plays several roles involving physiological mechanisms, such as hydrolysis of nutrients from food, blood clotting, the immune response, signal transduction, cell–cell recognition, hormonal activation, stress responses, inflammation and cell development (O'Connell et al.,

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2006). Moreover, proteolytic enzymes are parasitic and microbial digestive organisms' main component thereby enabling their invasion of host tissues and migration within them (El-Ebiarie and Taha, 2012).

Serine proteases are characterised by having a nucleophilic serine residue in their active site (*i.e.* the catalytic triad) containing histidine, asparagine and serine amino acids (Hedstrom, 2002). These proteases have extensive substrate specificity, this being consistent with the biological role played in most insects, such as larval establishment and providing the larvae with nutrients (Casu et al., 1996; Tabouret et al., 2003; Young et al., 1996). They have been found in the digestive tract of species belonging to the Lepidoptera and Coleoptera orders (Telang et al., 2005; Vinokurov et al., 2006) and also in some Diptera, such as *Oxysarcodexia thornax*, *Hypoderma lineatum*, *Oestrus ovis*, *Dermatobia hominis*, *Musca domestica*, *Lucilia cuprina*, *Crysomyia bezziana*, *Chrysomya megacephala* and *L. sericata* (Angulo-Valadez et al., 2007; Casu et al., 1994; Cuervo et al., 2008; Chambers et al., 2003; Lemos and Terra, 1992; Muharsini et al., 2000; Otranto, 2001; Pires et al., 2007; Taha et al., 2010). Trypsin and chymotrypsin are the most common enzymes in the serine protease family; they have been characterised and can mainly be found in insects' digestive systems (Mazumdar-Leighton and Broadway, 2001).

Differently to the above-mentioned species, no information regarding proteases present in *S. magellanica* blowfly ES is currently available in the pertinent literature, even though this species shares characteristics classifying it as potentially useful in larval therapy. The present study has thus been aimed at reporting the identification and characterisation of the enzymes found in *S. magellanica* larvae ES products and comparing them to those found in the related species, *L. sericata*.

2. Materials and methods

2.1. Collecting the flies and breeding the larvae

The *S. magellanica* laboratory colony used in this study was established in the Universidad del Rosario's Medical and Forensic Entomology laboratory. The adult forms were kept in 45 × 45 × 45 cm Gerberg cages at 24 °C, 70% relative humidity and 12 h light photoperiod. The flies were fed on pig's liver and a carbohydrate-rich source (30% sucrose) supplemented with vitamin B12 (Broderick et al., 2006).

Eggs laid by the colony established with flies from the *S. magellanica* species were placed in Petri dishes containing decomposing pig's liver or egg-powdered milk synthetic medium as alimentary substrates. First-, second- and third-instar larvae, as determined by the number of spiracle gaps (Florez and Wolff, 2009), were taken for extracting larval ES and providing continuity for the colony. *L. sericata* larvae were bred in the same conditions as described above and used as control in later assays. These larvae were taken from a previously-established colony (Rueda et al., 2010).

2.2. Extracting larval excretions and secretions (ES)

The ES were prepared following the methodology described by El-Ebiarie and Taha (2012). Briefly, 200 from each first-, second- and third-instar larvae from *S. magellanica* and *L. sericata* colonies were collected and washed for 5 min with a 0.5% sodium hypochlorite, 5% formaldehyde solution and sterile water plus antibiotics. The larvae were then incubated at 37 °C for 1 h in the dark in 1 mL MilliQ sterile water. The sample was spun at 14,000 × *g* for 5 min at 4 °C and the supernatant (containing the ES product) was quantified using a BCA (bicinchoninic acid) kit (Thermo Scientific), with BSA (bovine serum albumin) as standard for the reference curve and stored at −20 °C until use.

2.3. Separating ES by SDS-PAGE

S. magellanica ES were run on 14% SDS-PAGE in reducing conditions. Briefly, 50 μg ES from each larval stage fed on decomposing pig's liver or on egg-powdered milk synthetic medium were homogenised in SDS reducing buffer (0.5 M Tris–HCl pH 6.8, 25% glycerol, 10% SDS, 0.5% bromophenol blue and 5% β-mercaptoethanol), heated at 60 °C for 5 min and then run on SDS-PAGE at 70 V. The gels were stained with Coomassie blue (0.1% Coomassie blue, 40% methanol and 10% acetic acid) or Simply Blue Safe Stain for 1 h at room temperature and then incubated with a destaining solution (40% methanol (v/v) and 5% acetic acid (v/v)) or distilled water for 1 day for visualising the protein profile. A sample of *L. sericata* ES fed in line with the aforementioned parameters was also evaluated.

2.4. Zymographic assays

S. magellanica ES enzymatic activity was assessed on gels co-polymerised with 0.1% gelatin, according to a previously described methodology (Williams and Coombs, 1995). Three micrograms of *S. magellanica* larval ES and 1.5 μg *L. sericata* ES were incubated at 37 °C for 16 h at 10 mM, 30 mM and 50 mM final concentration, separately, with each of the following protease inhibitors: phenylmethylsulfonyl fluoride (PMSF), tosyl lysyl chloromethyl ketone (TLCK), tosyl phenylalanyl chloromethyl ketone (TPCK), iodoacetamide, ethylene-diamine-tetracetic acid (EDTA) and leupeptin. ES incubated without any inhibitor was used as positive control whilst negative control involved using ES previously heated at 60 °C for 1 h. The samples were homogenised in non-denaturing buffer (0.5 M Tris–HCl pH 6.8, 25% glycerol, 10% SDS and 0.5% bromophenol blue) for 14% polyacrylamide gel electrophoresis and then run at 80 V. The gels were then incubated with re-naturing buffer (2.5% Triton X-100) for 30 min at room temperature with constant shaking. After excess Triton had been removed by washing with distilled water, the gels were incubated for 30 min with developing buffer containing 50 mM Tris-base, 30 mM Tris–HCl, pH 7.4, 0.2 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij 35 and then left with constant shaking for 16 h at 37 °C using fresh buffer. The gels were then stained with Coomassie blue for 2 h and destained with destaining solution (10% v/v methanol and 5% v/v acetic acid) for 24 h. Protease molecular mass was calculated by comparison with the mobility of a commercial molecular mass standard. All results were derived from five independent experiments carried out in triplicate.

2.5. Serine-protease enzymatic assay

S. magellanica ES tryptic and chymotryptic activity was evaluated using BAPNA and SAPNA substrates, according to that reported by Muharsini et al. (2000). The substrates were prepared in buffer containing 100 mM Tris–HCl pH 7.8 and 20 mM $CaCl_2$. 40 μL of each substrate (4 mM BAPNA or 3.5 mM SAPNA) plus 150 μL buffer (pH 7.5, which has been shown to be the ideal pH for determining proteolytic activity in earlier studies (Cuervo et al., 2008; Saboia-Vahia et al., 2013; Xavier et al., 2005) and a closely related pH, 7.8) and 10 μg ES alone or previously incubated for 10 min with 10 mM PMSF, TLCK or TPCK seeded per well in 96-well polysorb plates. The resulting mixtures were incubated for 1 h at 37 °C and optical density (OD) was measured at 405 nm using an MJ ELISA Multiskan reader. The values for each sample were calculated by subtracting the OD value from the control value (ES alone). These assays were done in triplicate.

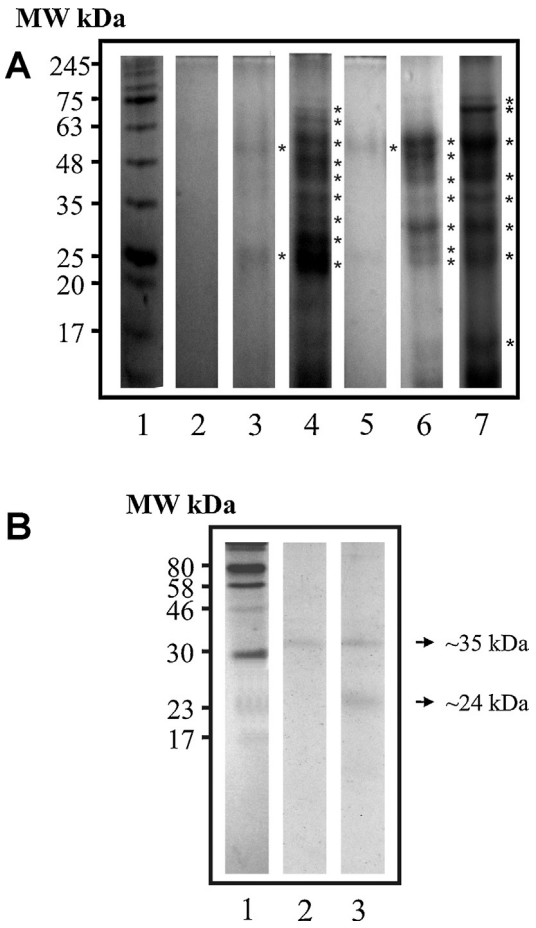

Fig. 1. The ES protein profile for *S. magellanica* and *L. sericata*. (A) protein profile of the three *S. magellanica* and *L. sericata* larval stages. Lane 1: molecular weight marker. Lanes 2 and 5 show the first larval stage from *S. magellanica* and *L. sericata*, respectively. Lanes 3 and 6 show the second *S. magellanica* and *L. sericata* larval stage, respectively. Lanes 4 and 7 show the third larval stage from *S. magellanica* and *L. sericata*, respectively. (B) Protein profile of third stage maggots fed on a synthetic diet. Lane 1: molecular weight marker. Lanes 2 and 3 *S. magellanica* and *L. sericata* larval ES, respectively.

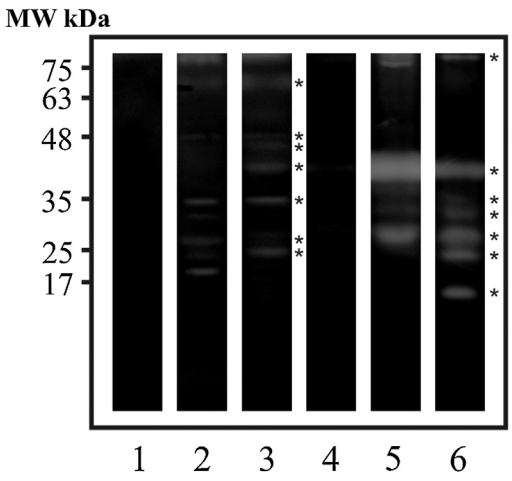

Fig. 2. Enzymatic profile of three larval stages from *S. magellanica* and *L. sericata*. Lanes 1–3 show the degradation pattern of the first, second and third larval stage of *S. magellanica*, respectively. Lanes 4–6 show the degradation pattern of the first, second and third *L. sericata* larval stages, respectively.

2.6. Statistical analysis

An ANOVA parametric test was used for analysing the differences between *S. magellanica* species ES inhibition assays. Such assays were compared to the control group using Dunnett and Bonferroni's tests. A 95% confidence interval was used for confirming the proposed hypothesis.

3. Results

3.1. S. magellanica larval ES protein profile

Total protein analysis involving SDS-PAGE of ES products from *S. magellanica* first-, second- and third-larval instar fed on the pig's liver diet revealed no bands in the first stage (Fig. 1, lane 2), whilst a weak band was observed for *L. sericata* (Fig. 1, lane 5: shown with asterisk). The second stage of *S. magellanica* revealed weak bands at ~24 kDa and ~ 49 kDa (Fig. 1, lane 3: shown with asterisks) whilst bands at ~23 kDa, ~25 kDa, ~27 kDa, ~35 kDa, ~40 kDa, ~49 kDa and ~52 kDa were observed in *L. sericata* (Fig. 1, lane 6). Dominant ~23 kDa, ~25 kDa, ~28 kDa, ~35 kDa, ~45 kDa, ~48 kDa, ~52 kDa, ~65 kDa and 69 kDa bands for *S. magellanica* (shown with asterisks) were observed in third larval stage (Fig. 1, lane 4), and ~15 kDa, ~24 kDa, ~27 kDa, ~35 kDa, ~45 kDa, ~52 kDa, ~69 kDa and ~75 kDa bands were observed for *L. sericata* (Fig. 1, lane 7: shown with asterisks). Taking into account that the third larval instar showed a greater number of bands in both species, the ES protein profile for larvae fed on a synthetic diet was thus analysed in this stage. The profile revealed just a few bands: a ~35 kDa in *S. magellanica* and ~35 kDa and ~24 kDa bands in *L. sericata* (Fig. 1B, lanes 2 and 3, respectively).

3.2. Identifying S. magellanica ES proteases

Comparing ES product enzymatic activity amongst the three *S. magellanica* and *L. sericata* larval stages promoted degradation of the gelatine substrate; however, distinct proteolytic profiles were observed between species (Fig. 2). The first stage of *S. magellanica* and *L. sericata* did not have proteolytic activity (Fig. 2, lanes 1 and 4) whilst proteolysis patterns were observed between second and third larval stage ES from each species (Fig. 2, lanes 2, 3, 5 and 6). Prominent bands in *S. magellanica* third larval stage were observed at ~25 kDa, ~28 kDa, ~35 kDa, ~45 kDa, ~47 kDa, ~48 kDa and ~65 kDa (Fig. 2, lanes 2 and 3: shown with asterisks) whilst activity for *L. sericata* ES was observed at ~15 kDa, ~24 kDa, ~27 kDa, ~ 33 kDa, ~35 kDa, ~45 kDa and higher than ~75 kDa (Fig. 2, lane 6).

Protease activity was characterised by incubating the ES from the third stage larvae from both species with serine-, cysteine- and metallo-protease inhibitors at 37 °C for 16 h. The *S. magellanica* assay showed that ~25 kDa band intensity became totally reduced when using PMSF or EDTA inhibitors at their highest concentration (Fig. 3A, lanes 2 and 4) compared to control (Fig. 3A, lane 1), thereby suggesting that such band mainly consisted of serine- and metallo-proteases. No degradation was observed in the control lane (Fig. 3A, lane 6), indicating enzyme denaturation (60 °C). The proteolytic activity pattern observed for the proteases present in *L. sericata* ES (Fig. 3B) showed bands of ~15 kDa,~24 kDa, ~27 kDa, ~35 kDa, ~45 kDa and ~52 kDa which were totally reduced using PMSF (Fig. 3B, lane 2) and others became partially reduced with iodoacetamide, EDTA or leupeptine (Fig. 3A, lanes 3–5). It was difficult to characterise *S. magellanica* ~28 kDa, ~35 kDa, 45 kDa, 52 kDa and ~65 kDa bands, taking into account that they became degraded in the incubation conditions used in the assay (Fig. 3A, lane 1).

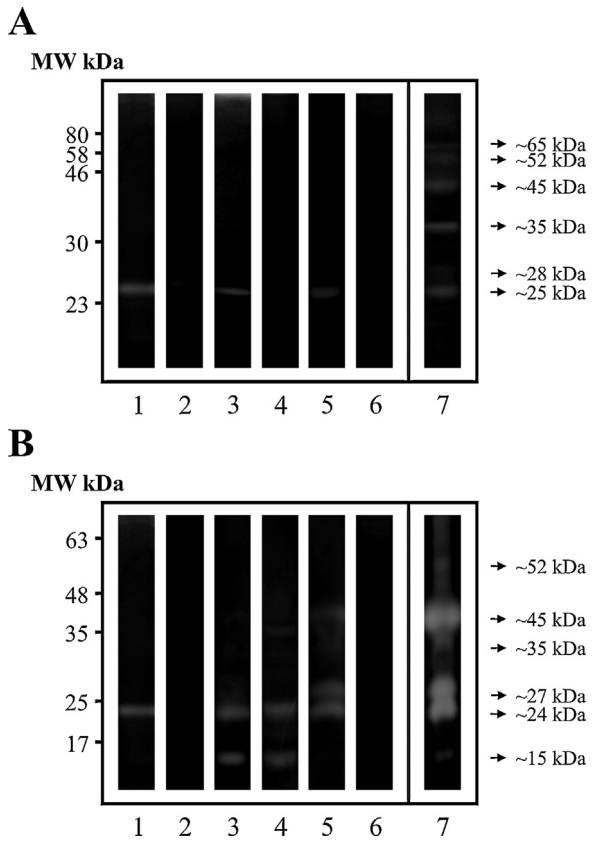

Fig. 3. The effect of *S. magellanica* and *L. sericata* ES proteolytic activity on gelatine substrate. (A) *S. magellanica* larval ES; (B) *L. sericata* larval ES. Lane 1 shows the ES for each species incubated at 37 °C without protease inhibitors. Lanes 2–5 show the ES for each species incubated with PMSF, iodoacetamide, EDTA and leupeptine, respectively. Lane 6 indicates the ES heated at 60 °C for 1 h (control). Lane 7 shows *S. magellanica* and *L. sericata* ES without incubation at 37 °C.

3.3. *S. magellanica* larval ES serine-protease characterisation

Gelatine degradation (inhibited using serine protease specific inhibitors: TLCK for trypsin and TPCK for chymotrypsin) was initially evaluated in zymogram assays (Fig. 4); total hydrolysis was observed when the sample was incubated with TLCK or TPCK (Fig. 4, lanes 2 and 3), differently to control (Fig. 4, lane 1). A further assay was performed which involved using trypsin- (BAPNA) and

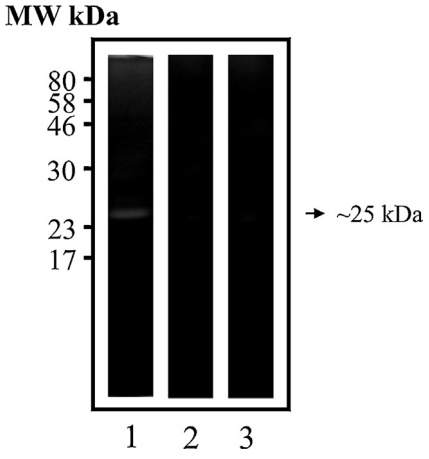

Fig. 4. Zymogram showing the inhibition of *S. magellanica* ES proteolytic activity. Lane 1: larval ES. Lanes 2 and 3 show the ES incubated with TLCK and TPCK, respectively.

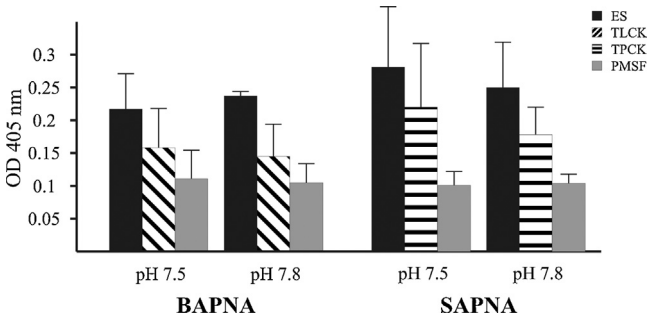

Fig. 5. Serine protease characterisation of *S. magellanica* ES using BAPNA and SAPNA substrates at different pH, in the presence of TLCK (bar with diagonal lines) and TPCK inhibitors (bar with horizontal lines). The black bar indicates ES whilst the grey bar shows inhibition control using PMSF. Statistically significant differences between ES and TLCK, and between ES and PMSF, were observed at pH 7.8 ($p = 0.01$), whilst there was no difference at pH 7.5 because of less alkalinity ($p = 0.47$). No statistically significant difference between TPCK and ES was observed ($p = 0.67$ at pH 7.5; $p = 0.29$ at pH 7.8). A statistically significant difference ($p = 0.03$) was also seen between PMSF and ES on SAPNA substrate at pH 7.8.

chymotrypsin-specific substrates (SAPNA) for evaluating the type of serine proteases present in *S. magellanica* larval ES (Fig. 5). Such analysis revealed a statistically significant difference between the proteolytic activity of ES incubated with TLCK and control at pH 7.8 ($p = 0.05$) whilst there was no difference at pH 7.5 ($p = 0.47$). Only a statistically significant difference was observed between the larval ES incubated with such inhibitor and the control at pH 7.8 ($p = 0.01$) when using PMSF in the assays. There was no statistically significant difference between the treatment of ES incubated with TPCK and control at any pH ($p = 0.67$ at pH 7.5; $p = 0.29$ at pH 7.8). A statistically significant difference was found at pH 7.8 ($p = 0.03$) when evaluating the effect of PMSF on the SAPNA substrate compared to control (ES). The foregoing results suggested that the TLCK and PMSF inhibitors had greater activity at pH 7.8.

As there was no significant difference in TPCK activity on SAPNA substrate, the percentage of TLCK protease inhibitor activity on *S. magellanica* ES was compared to that of the BAPNA substrate at pH 7.8 (summarised in Table 1). As shown, ES proteolytic activity was less inhibited at 45% using PMSF on the treated ES whilst greater than 61% values were found when using TLCK; such discrepancy could have been due to each inhibitor's substrate specificity. The above data thus support the idea that ES from *S. magellanica* species mainly contain trypsin-like proteases.

4. Discussion

Larval therapy has been an important approach to healing necrotic wounds caused by pressure, venous/arterial insufficiency or diabetes (Telford et al., 2011). It has been shown that once the larvae have been applied to a wound, they trigger processes that help to debride, eliminate bacteria and stimulate the formation of granulation tissue by means of releasing ES protein products (Horobin et al., 2003).

The Coomassie-stained larval ES gels demonstrated that the second and third larval stage had higher protein production than the

Table 1
The effect of *S. magellanica* ES trypsin-like serine proteases on BAPNA substrate hydrolysis. Statistically significant differences were observed between ES and TLCK, and between ES and PMSF ($p = 0.01$).

Inhibitor	pH	Mean OD ± SD	Inhibition (%)
Control	7.8	0.237 ± 0.007	0
TLCK		0.145 ± 0.049	61.1
PMSF		0.105 ± 0.029	44.3

SD: standard deviation; OD: Optical density.

first in both species (Fig. 1A). On the other hand, the proteolytic profile from all analysed stages showed that the third larval instar had a greater number of bands in both species (Fig. 2), this being consistent with those obtained from other species such as *D. hominis*, *A. albopictus* and *O. ovis* (Brant et al., 2010; Saboia-Vahia et al., 2013; Tabouret et al., 2003). It has been reported that the higher proteolytic activity in second and third larval stage could be associated with the highest increase in weight after their molting (Cepeda-Palacios et al., 1999), when the larvae had acquired about 45% of their average mature weight. The ES from *L. sericata* third larval stage has been shown to display significant antibacterial activity in larval therapy in several studies (Arora et al., 2011; Bexfield et al., 2004; Cazander et al., 2009). It has also been described that this stage produces greater ES yield (Bexfield et al., 2004). Cazander et al. (2009), in a study about the influence of maggot excretions on *Pseudomonas aeruginosa* biofilm formation on different biomaterials, have shown that third-instar larvae produce much more ES than first instar larvae; they used this argument to explain why ES third-instar larvae were more effective in reducing biofilm. They also assumed that ES first instar larvae composition differed from that of ES third-instar larvae, effectively coinciding with our results.

The *S. magellanica* ES protein profile was characterised by having bands from ~23 kDa to ~63 kDa (Fig. 1A, lane 4), this being similar to the results reported for several species such as *L. sericata* (Chambers et al., 2003; Schmidtchen et al., 2003), *C. megacephala* (El-Ebiarie and Taha, 2012; Taha et al., 2010), *L. cuprina* (Young et al., 1996), *C. bezziana* (Muharsini et al., 2000) and *O. ovis* (Tabouret et al., 2003).

Once the ES profile for *S. magellanica* and *L. sericata* larvae fed on a synthetic diet had been compared to that for those fed on a natural diet, it was found that ES from those fed on a natural diet contained a greater amount of proteins (Fig. 1B), thereby coinciding with previous reports where it was described that larvae fed on a natural diet (especially decomposing tissue or bacteria-rich environments) secrete a greater amount of proteins (Andersen et al., 2010; Barnes and Gennard, 2011; Bexfield et al., 2004).

The *S. magellanica* ES enzyme degradation pattern was clearly seen at ~25 kDa (Fig. 3A). Other authors have found enzyme degradation in a range close to that for the band found here, as in *L. sericata* (Chambers et al., 2003), *L. cuprina* (Young et al., 1996), *C. bezziana* (Muharsini et al., 2000) and *O. ovis* (Angulo-Valadez et al., 2007). By contrast, proteolytic activity ranging from ~220 kDa to ~168 kDa has been reported for the *D. hominis* species (Brant et al., 2010); such difference could have been due to the larvae's physiological requirements for actively invading the host's skin and migrating in it (Brant et al., 2010), thereby contributing towards haemoglobin, serum albumin and collagen hydrolysis (Pires et al., 2007). Interestingly, a strong decrease in proteolytic activity was observed for some bands after incubation at 37 °C (Fig. 3A, lane 1 and 7); this might have been due to the enzymes' thermal denaturation, as reported in a recent study (Saboia-Vahia et al., 2013).

The main proteases detected in the larvae from other Diptera species are serine proteases, these being the main low molecular weight molecules involved in proteolytic activity (Casu et al., 1996; Cuervo et al., 2008; Lemos and Terra, 1992; Muharsini et al., 2000; Pires et al., 2007) and playing a very important role in healing wounds (Telford et al., 2011). Two main types of enzyme in the serine protease family, such as trypsin and chymotrypsin, have been identified in the species *L. cuprina* (Casu et al., 1996; Sandeman et al., 1990), *C. bezziana* (Muharsini et al., 2000), *H. lineatum* (Otranto, 2001) and *T. molitor* (Elpidina et al., 2005). Serine and metallo-proteases identified in *S. magellanica* had hydrolysis reduction on gelatine substrate incubating ES with PMSF and EDTA, respectively (Fig. 3A, lanes 2 and 4). Using either TLCK or TPCK inhibitors for characterising serine proteases by zymogram revealed that *S. magellanica* ES contained trypsin-like and chymotrypsin-like proteases (Fig. 4). The specific substrate assay revealed the highest reduction for the TLCK inhibitor, suggesting that the ES contained mainly trypsin-like proteases (Fig. 5). It has been shown that metallo-proteases are responsible for cutaneous penetration in *Ancylostoma* sp. (Hotez et al., 1990) and *Strongyloides stercoralis* (Brindley et al., 1995). They have also been implicated in removing damaged proteins in a wound, in cell migration and new blood vessel development (Lobmann et al., 2005). A previous report has shown that trypsin plays an important role in receptor-mediated activation of cell proliferation or cytokine secretion within a wound (Horobin et al., 2003), whilst another study has demonstrated that chymotrypsin is the most important serine protease in degrading some extracellular matrix components, such as fibronectin, laminin and type I and III collagen (Chambers et al., 2003).

When evaluating *S. magellanica* ES proteolytic role regarding BAPNA medium using different pH, no significant differences were found concerning its activity ($p = 0.63$). Previous reports have shown that ES from *L. sericata* (Chambers et al., 2003), *O. thornax* (Cuervo et al., 2008), *D. hominis* (Pires et al., 2007), *O. ovis* (Tabouret et al., 2003) and *C. bezziana* (Muharsini et al., 2000) species are more active at alkaline pH (7.5–9.5); this could be a mechanism used by the larvae for promoting the debridement of a wound involving ammonium excretion into the medium resulting in a wound's pH increasing (Chambers et al., 2003) or for larval infestation in parasitic insects (Cuervo et al., 2008). Some studies have also reported the presence of trypsin-like proteases which are highly active at alkaline pH but which are not activated by calcium ions and have similar properties to those of other species producing miasis, such as *L. cuprina* (Casu et al., 1994), *C. bezziana* (Muharsini et al., 2000), *D. hominis* (Brant et al., 2010) and *T. molitor* (Elpidina et al., 2005). Likewise, the results described here have shown that the main *S. magellanica* ES proteases (trypsin-like) have optimum proteolytic activity at an alkaline pH.

5. Conclusions

The present work has characterised *S. magellanica* larval excretion and secretion products for the first time; it has shown that ES products of this blowfly species (mainly found throughout South-America) consist mainly of trypsin-like serine peptidases, according to zymographic profiles and enzymatic assays. Studies involving species from the Calliphoridae family have led to new perspectives regarding the use of larvae for treating necrotic wounds. *L. sericata* is currently the species being most used in this respect, whilst a few other species had been evaluated regarding their biotherapeutic potential. The present study reinforces further experiments aimed at evaluating the biological effect induced by *S. magellanica* ES in *in vitro* cultures, as well as assays using larvae in induced wounds in animal models which are currently being carried out by our research group.

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