



Defensins from the tick *Ixodes scapularis* are effective against phytopathogenic fungi and the human bacterial pathogen *Listeria grayi*

Miray Tonk, Alejandro Cabezas Cruz, James J. Valdes, Ryan Om Rego, Tereza Chrudimská, Martin Strnad, Radek Sima, Lesley Bell-Sakyi, Zdeněk Franta, Andreas Vilcinskis, et al.

► To cite this version:

Miray Tonk, Alejandro Cabezas Cruz, James J. Valdes, Ryan Om Rego, Tereza Chrudimská, et al.. Defensins from the tick *Ixodes scapularis* are effective against phytopathogenic fungi and the human bacterial pathogen *Listeria grayi*. *Parasites & Vectors*, 2014, 7 (554), 10.1186/s13071-014-0554-y . hal-02639796

HAL Id: hal-02639796

<https://hal.inrae.fr/hal-02639796>

Submitted on 28 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/267448311>

Defensins from the tick *Ixodes scapularis* are effective against phytopathogenic fungi and the human bacterial...

Article in *Parasites & Vectors* · October 2014

DOI: 10.1186/PREACCEPT-8265353130225079

CITATIONS

11

READS

135

12 authors, including:



Ryan O. M. Rego

Biology Centre, ASCR, v.v.i., Institute of Paras...

73 PUBLICATIONS 407 CITATIONS

[SEE PROFILE](#)



Tereza Chrudimska

The Czech Academy of Sciences

5 PUBLICATIONS 69 CITATIONS

[SEE PROFILE](#)



Libor Grubhoffer

Biology Centre, Czech Acad. Sci., Institute of ...

208 PUBLICATIONS 2,466 CITATIONS

[SEE PROFILE](#)



Mohammad Rahnamaeian

Fraunhofer Institute for Molecular Biology an...

36 PUBLICATIONS 311 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



The structure of tick-host-pathogens phylogenetic and environmental interactions [View project](#)



Eugenics [View project](#)

RESEARCH

Open Access

Defensins from the tick *Ixodes scapularis* are effective against phytopathogenic fungi and the human bacterial pathogen *Listeria grayi*

Miray Tonk^{1,2}, Alejandro Cabezas-Cruz^{3,4}, James J Valdés¹, Ryan OM Rego¹, Tereza Chrudimská^{1,2}, Martin Strnad^{1,2}, Radek Šíma¹, Lesley Bell-Sakyi⁵, Zdeněk Franta⁶, Andreas Vilcinskas^{6,7}, Libor Grubhoffer^{1,2*} and Mohammad Rahnamaeian^{6,7*}

Abstract

Background: *Ixodes scapularis* is the most common tick species in North America and a vector of important pathogens that cause diseases in humans and animals including Lyme disease, anaplasmosis and babesiosis. Tick defensins have been identified as a new source of antimicrobial agents with putative medical applications due to their wide-ranging antimicrobial activities. Two multigene families of defensins were previously reported in *I. scapularis*. The objective of the present study was to characterise the potential antimicrobial activity of two defensins from *I. scapularis* with emphasis on human pathogenic bacterial strains and important phytopathogenic fungi.

Methods: Scapularisin-3 and Scapularisin-6 mature peptides were chemically synthesised. *In vitro* antimicrobial assays were performed to test the activity of these two defensins against species of different bacterial genera including Gram-positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Listeria spp.* as well as Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* along with two plant-pathogenic fungi from the genus *Fusarium*. In addition, the tissue-specific expression patterns of Scapularisin-3 and Scapularisin-6 in *I. scapularis* midgut, salivary glands and embryo-derived cell lines were determined using PCR. Finally, tertiary structures of the two defensins were predicted and structural analyses were conducted.

Results: Scapularisin-6 efficiently killed *L. grayi*, and both Scapularisin-3 and Scapularisin-6 caused strong inhibition (IC₅₀ value: ~1 µM) of the germination of plant-pathogenic fungi *Fusarium culmorum* and *Fusarium graminearum*. Scapularisin-6 gene expression was observed in *I. scapularis* salivary glands and midgut. However, Scapularisin-3 gene expression was only detected in the salivary glands. Transcripts from the two defensins were not found in the *I. scapularis* tick cell lines ISE6 and ISE18.

Conclusion: Our results have two main implications. Firstly, the anti-*Listeria* and antifungal activities of Scapularisin-3 and Scapularisin-6 suggest that these peptides may be useful for (i) treatment of antibiotic-resistant *L. grayi* in humans and (ii) plant protection. Secondly, the antimicrobial properties of the two defensins described in this study may pave the way for further studies regarding pathogen invasion and innate immunity in *I. scapularis*.

Keywords: Antimicrobial peptide, Defensin, *Listeria grayi*, *Fusarium spp*, *Ixodes scapularis*, Tick cell line

* Correspondence: liborex@paru.cas.cz; mohammad.rahnamaeian@agrar.uni-giessen.de

¹Biology Centre of the AS CR, Institute of Parasitology, Branišovská 31, 37005 České Budějovice, Czech Republic

⁶Fraunhofer Institute for Molecular Biology and Applied Ecology, Department of Bioresources, Winchester Strasse, D-35394 Giessen, Germany

Full list of author information is available at the end of the article

Background

The blacklegged tick *Ixodes scapularis* is an important vector of pathogens such as *Borrelia burgdorferi*, *Babesia microti* and *Anaplasma phagocytophilum* that cause important diseases in humans and animals. In response to pathogen infection, ticks develop a complex system of defence that involves elements of the innate immune system [1]. One of the most important features of the tick immune response is the rapid synthesis and release of antimicrobial peptides (AMPs) [2]. Amongst the naturally-occurring AMPs, the small defensin peptides are the most studied and widely-characterised class of antimicrobial peptides from several tick species [2-4]. Mature defensins are cyclic peptides possessing a pattern of six paired cysteine residues in their primary structure with three or four disulphide bridges, forming a conserved cysteine-stabilised α -helix and β -sheet (CS $\alpha\beta$) motif crucial for their antimicrobial activity [1,5]. Most defensins are cationic; however some anionic defensins have been reported [6-8]. Two multigene families of defensins, Scapularisin and Scasin, were reported in *I. scapularis*, both of them presenting high phylogenetic and structural diversity [5]. A functional domain of one *I. scapularis* defensin, Scapularisin-20, was functionally characterised and found to show activity against Gram-negative (Gram-) and Gram-positive (Gram+) bacteria [5]. However, tick defensins have a wide spectrum of antimicrobial activity, including antibacterial, antifungal, antiprotozoal and antiviral activities; thus defensins are excellent candidates to tackle the increasing problem of drug resistance seen in microbes and fungi [9-11].

Listeria spp. are small Gram+, non spore-forming, facultative anaerobic bacteria widely distributed in the environment [12-14]. The *Listeria* genus consists of 10 species: *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria fleishmannii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri*, *Listeria martini*, *Listeria rocourtiae*, *Listeria weihenstephanensis* and *Listeria grayi* [15-20]. In addition to the major human pathogen *L. monocytogenes* [13]. Occasional human infections have been reported with *L. ivanovii* [21-23], *L. grayi* [24], *L. seeligeri* and *L. innocua* [25,26]. There is also some evidence suggesting that strains of *L. innocua* are occasionally pathogenic to deer, cattle and sheep [27]. *Listeria* species have also been isolated from a variety of food products, including commercially prepared ready-to-eat foods and from food-processing environments [14]. Recently, a human stem cell transplant recipient was found to be infected with a vancomycin-resistant strain of *L. grayi* [28]. *Fusarium culmorum* is a ubiquitous soil-borne fungus and plant pathogen capable of causing ear and root rot in different small-grain cereals, in particular wheat and barley. Contamination with *F. culmorum* causes reduction in plant growth and significant yield and quality losses.

In addition, the fusarium infection is concomitant with mycotoxin contamination [29,30]. *Fusarium graminearum* is another very important causative agent of Fusarium head blight (FHB) of small grain cereals like wheat and barley and of ear rot in maize [31] and other cereals worldwide [32,33].

Herein, we examined and characterised the activity, where present, of synthetic Scapularisin-3 and Scapularisin-6 mature peptides against the Gram + bacteria *L. grayi*, other *Listeria* spp., *Staphylococcus aureus* and *Staphylococcus epidermidis*, the Gram- bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, and the fungal pathogens *F. culmorum* and *F. graminearum*. Additionally, we determined the transcriptional expression pattern of these defensins in the salivary glands and midgut of *I. scapularis* ticks and in two *I. scapularis* embryo-derived cell lines, as well as carrying out a structural analysis of the two defensins.

Methods

Defensin sequences and preparation of synthetic Scapularisins

Using publicly available sequence data [5], 38 amino acids of the mature peptides of each of Scapularisin-3 (Genbank accession number: EEC13914) and Scapularisin-6 (Genbank accession number: EEC08935) were chemically synthesised with $\approx$ 95% purity (Peptide 2.0, USA). Lyophilised peptides were stored at -20°C until use.

Antibacterial assays

Concentrations of the peptides ranging from 0.03 to 250 μM were used to test antibacterial activity and MIC (minimum inhibitory concentration) of the peptides against Gram + bacterial species including *L. fleishmannii* (DSM 24998), *L. grayi* (DSM 20601), *L. marthii* (DSM 23813), *L. innocua* (DSM 20649), *L. welshimeri* (DSM 20650), *L. seeligeri* (DSM 20751), *L. rocourtiae* (DSM 22097), *S. aureus* (DSM 2569) and *S. epidermidis* (DSM 3269) as well as the Gram- bacteria *E. coli* (D31) and *P. aeruginosa* (DSM 50071). The assays were performed in either Brain Heart Infusion Broth (BHIB) medium in case of *Listeria* spp. or Tryptic Soy Broth (TSB) (Roth, Karlsruhe, Germany) for others, in 384-well plates (Griener Bio One, Frickenhausen, Germany). Bacteria in the mid-logarithmic phase were used for growth inhibition assays. The initial optical density (OD), at 600 nm, for *Listeria* spp. was set to 0.01 and for the rest of the bacteria to 0.001 to ensure full contact of each bacterial cell with defensins added to the suspensions. Changes in OD were monitored at 20 min intervals over a 24 h period using an EonTM Microplate Spectrophotometer (BioTel Instruments, VT, USA). For each assay, a medium-only control culture was included. Antimicrobial activity of the peptides was tested against all bacteria and the assays were repeated for confirmation.

(All microorganism strains were available at the Fraunhofer Institute for Molecular Biology and Applied Ecology, Department of Bioresources).

Antifungal assays

Fungicidal activities of synthetic peptides were determined using an inhibition assay reported previously [34]. *F. culmorum* and *F. graminearum* strain 8/1 [35] were cultured in the dark on Nirenberg Synthetic Nutrient Agar (SNA) medium at 18°C for 1–2 weeks [36]. Briefly, *F. culmorum* and *F. graminearum* were incubated with different concentrations of the peptides from 0.1 µM to 20 µM at room temperature for 24 h under humid conditions. Spore germination and growth were monitored using an inverted microscope Motic AE21 (Motic, China). The IC₅₀ values were recorded when only 50% of the spores germinated in the solution.

Ticks and tick organ collection

I. scapularis ticks were provided by the Institute of Parasitology, Academy of Sciences of the Czech Republic. Uninfected ticks were reared for several generations in the animal facilities of the Institute and fed on adult guinea pigs that were raised to be free of infection under the hygiene regulations of the Central Commission for the Protection of Animals (§21, section 3e, the Animal Protection Law of the Czech Republic No. 246/1992 sb., ethics approval number 137/2008). Ticks were fed on adult guinea pigs and ten partially engorged (6 days post attachment) female *I. scapularis* were collected for further analysis. For salivary glands and midgut collection, the ticks were affixed to the bottom of a Petri dish with wax, submerged in PBS, incised along the dorso–lateral margin and the dorsal integument was removed under a binocular microscope. The organs were excised, washed in PBS to remove excess blood, transferred into RNeasy lysis solution (Qiagen, USA) and kept at –80°C for later use (protocol modified from [37]).

Cell culture

The *I. scapularis* embryo-derived cell lines ISE6 and ISE18 [38,39] were maintained at 28°C in L-15 (Leibovitz) medium (Life technologies, USA) supplemented with 20% heat-inactivated foetal bovine serum, 10% tryptose phosphate broth, 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin B. Medium was changed weekly by removal and replacement of 3/4 of the medium volume and subcultures were carried out when required [40].

RNA isolation and cDNA synthesis

The salivary glands and midguts for RNA isolation were homogenised by passing through a 0.9 mm needle (20 gauge) fitted to a syringe. From the ISE6 and ISE18 *in vitro*

cultures, 5×10^6 cells were harvested. Total RNA isolation from salivary glands, midgut, ISE6 and ISE18 was performed using the NucleoSpin® RNA II kit (Macherey Nagel, Germany). A total of 60 µL RNase-free H₂O was used for elution. The RNA concentration and purity were determined by measuring the optical density at both 260 and 280 nm using a spectrophotometer NanoDrop® ND-1000 (Peqlab, Erlangen, Germany). After determination of RNA concentration and integrity, the samples were diluted using RNase-free H₂O, dispensed into aliquots of 10 µL each and frozen at –80°C until being used in a cDNA synthesis reaction. Single-strand cDNA was prepared using 1 µg of total RNA with random primers (0.2 µg per reaction) using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Germany) according to the manufacturer's protocol.

PCR

The oligonucleotide primers (Generi Biotech, Czech Republic) used for detecting expression of genes from different tissues and cells are shown in Table 1. Beta-actin (β-actin) was used as a control. Two independent PCR reactions were performed for each gene. For each PCR amplification, 1 µL of cDNA was used as the template in a 20 µL reaction mixture containing 20 pmol of each primer and 2× PCR Master Mix (Promega, USA). The reactions were conducted in an Eppendorf Mastercycler Personal (Eppendorf, Germany) with the following parameters: 5 min at 96°C, followed by 35 cycles at 96°C for 30 s, 55°C for 30 s and 72°C for 1 min. The final extension step was at 72°C for 10 min. PCR products were visualised by agarose gel electrophoresis.

Tertiary protein modeling

Predicted tertiary models of the mature peptides were generated using the Phyre2 server [41]. The predicted models were then refined via minimisation and the hydrogen-bond network optimised using the Schrodinger's Maestro Protein Preparation Wizard [42]. The electrostatic potentials for each structure were calculated using the implemented Poisson-Boltzmann equation in the Maestro software.

Table 1 Primers used in this study

Primer	Sequence
Sca3-F	5'-ATGAAGGTCGTTGGAATTGCTCTT-3'
Sca3-R	5'-TTATTTCTGGTAACAGGTGCAAGTTC-3'
Sca6-F	5'-ATGAGGGTCATTGCTGTTACCTTGA-3'
Sca6-R	5'-TTAGTTGTGGTAGCATGTGCACGTC-3'
Actin-F	5'-ATGTGTGACGACGAGGTTGCCGC-3'
Actin-R	5'-GTACAGCGACAGCACGGCCTGG-3'

Results

Scapularisin-6 possesses inhibitory activity against *L. grayi*

The antimicrobial activities of both defensins were evaluated against the Gram + bacteria *S. aureus*, *S. epidermidis* and seven *Listeria* spp., and the Gram- bacteria *E. coli* and *P. aeruginosa*. Of these, only *L. grayi* was susceptible to increasing concentrations of Scapularisin-6, being totally inhibited in the presence of 120 μ M Scapularisin-6 (Figure 1). No activity against any of the bacterial species used in the antimicrobial assays was detected for Scapularisin-3, tested at a range of concentrations up to 250 μ M. Antimicrobial assays using a combination of Scapularisin-3 and 6 were performed, however no additive effect was observed (data not shown).

Scapularisin-3 and Scapularisin-6 are potent antifungal agents

Additionally, we assessed the antifungal activity of Scapularisin-3 and Scapularisin-6 using two major phytopathogenic fungi, *F. culmorum* and *F. graminearum*. Both Scapularisin-3 and Scapularisin-6 showed high levels of antifungal activity. Scapularisin-3 totally inhibited germination of *F. culmorum* and *F. graminearum* spores at concentrations of, 0.5 and 1 μ M, respectively (Table 2; Figure 2). In case of Scapularisin-6, maximum activity was recorded at 1 μ M and 2 μ M against *F. culmorum* and *F. graminearum* (Table 2; Figure 2) respectively.

Biochemical properties and tertiary structure of Scapularisin-3 and Scapularisin-6

The primary structures of Scapularisin-3 and Scapularisin-6 are shown in Figure 3A. The deduced amino acid sequences of the prodefensins are 70 amino acids for Scapularisin-3 and 74 amino acids for Scapularisin-6. As

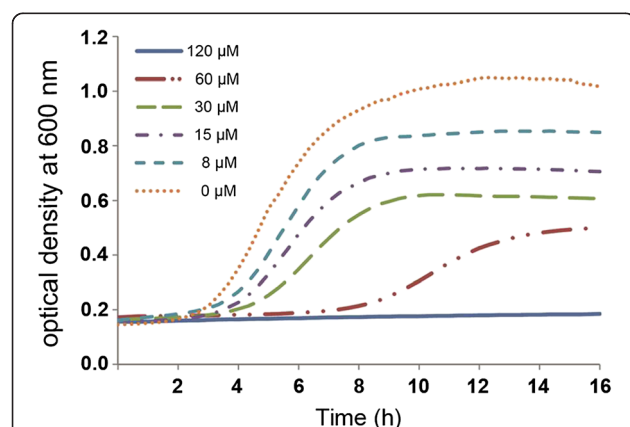


Figure 1 Antimicrobial activity of Scapularisin-6 against *L. grayi*. The figure represents bacterial growth curves at different concentrations of Scapularisin-6 peptide. Total growth inhibition was observed at 120 μ M of peptide concentration (blue line). The experiment was repeated with the same results.

Table 2 Antifungal activity of synthetic *I. scapularis* defensins

Peptide	IC ₅₀ value (μ M)	
	<i>F. culmorum</i>	<i>F. graminearum</i>
Scapularisin-3	0.5	1
Scapularisin-6	1	2

IC₅₀: Half maximal inhibitory concentration.

indicated in Figure 3A, the two prodefensins contain a furin motif, indicating putative enzymatic cleavage *in vivo* by the enzyme Furin. After cleavage, both mature proteins contain 38 amino acid residues. The mature peptides of Scapularisin-3 and Scapularisin-6 have predicted molecular weights of 4.3 kDa and 4.1 kDa, respectively. The tertiary structures of the *I. scapularis* defensins are similar and are conserved when compared to the NMR structure of the antibacterial defensin DEF-AAA from *Anopheles gambiae* ([43]; Figure 3B). The defensins depicted in Figure 3, however, are missing the archetypal disulphide bond that links both termini, and deletions in the α -core motif and insertions in the γ -core motifs are shorter. The overall backbone deviation does not exceed 3.2 Å and the deviation between the two *I. scapularis* defensins are <1 Å (Figure 3C). In Figure 3B we also show that the cationic electrostatic surface of Scapularisin-3 (pI 9.4) and Scapularisin-6 (pI 8.7) is mainly basic (blue); however, Scapularisin-6 possesses slightly more acidic residues (red). These acidic surfaces are within the α -core motif with the residues Thr10 and Ser13 in Scapularisin-3 substituted by Ala10 and Arg13 in Scapularisin-6 (GxCHxHC), indicated by the yellow arrowhead in Figure 3B.

Scapularisin-3 and Scapularisin-6 present different expression patterns

In order to determine the expression profiles of Scapularisin-3 and Scapularisin-6 in *I. scapularis* salivary gland, midgut and embryo-derived cell lines, RNA was extracted from these tissues and PCR amplification of transcripts of the Scapularisin-3, Scapularisin-6 and β -actin genes was performed. Scapularisin-6 gene expression was observed in both salivary glands and midgut (Figure 4). However, Scapularisin-3 gene expression was only observed in the salivary glands. No transcripts from either defensin were amplified from the ISE6 or ISE18 tick cell lines (Figure 4).

Discussion

I. scapularis presents one of the largest defensin families reported so far in ticks. This tick species presents two phylogenetically divergent groups of defensins named Scapularisin and Scasin, with 25 and 21 members, respectively [5]. Of the 25 Scapularisins, only the γ -core of Scapularisin-20 has been functionally characterised so

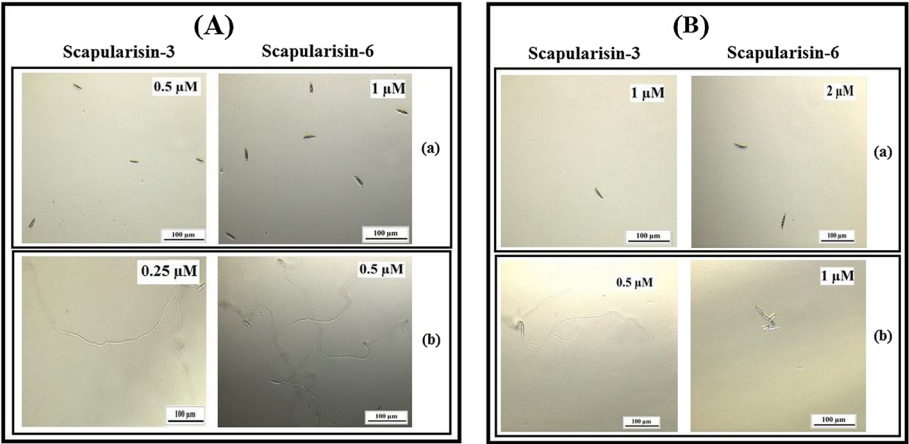


Figure 2 Antifungal activity of Scapularisin-3 and Scapularisin-6 against *Fusarium* spp. Images show (a) inhibition of spore germination and (b) spore germination (presence of hyphae) at different concentrations of Scapularisin-3 and Scapularisin-6. Pictures of the respective fungal cultures were taken after 24 hours of incubation of *F. culmorum* (A) and *F. graminearum* (B) with the peptides.

far, and it shows antimicrobial activity against Gram – and Gram + bacteria [5]. Thus a gap exists regarding the spectrum of biological functions of *I. scapularis* defensins. Our results expand the knowledge on the antimicrobial spectrum of *I. scapularis* defensins since we showed that Scapularisins are effective not only against the bacterium *L. grayi*, but also against fungi *F. culmorum* and *F. graminearum*. In addition, this is the first study showing the activity of tick defensins against the above microorganisms. The antibacterial activity of tick defensins has been reported to be mainly directed against Gram + bacteria, but

some isoforms were reported to also have activity against Gram- bacteria [44-49]. However, the activity of the defensins tested in this study seems to be rather specific, mainly against fungi. The γ -core of Scapularisin-3 and Scapularisin-6 differ in 5 amino acids (out of 14 that form the γ -core) with that of Scapularisin-20 (data not shown). Considering the importance of the γ -core in the biological function of defensins, sequence divergence may explain the differences in functions among these three defensins. In addition, in the present study we tested the activity of the mature Scapularisin-3 and Scapularisin-6 while Wang and Zhu, 2011 [5] tested only the

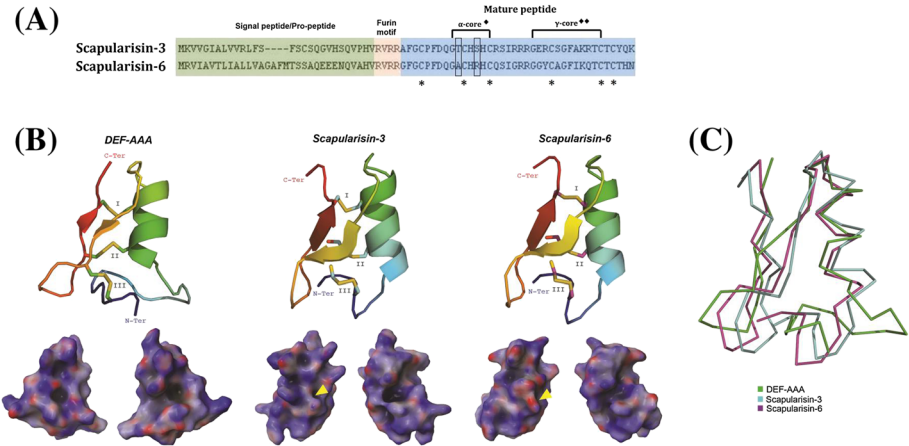


Figure 3 Biochemical properties and tertiary structure of Scapularisin-3 and Scapularisin-6. (A) Alignment of the primary structure of the defensins: represents Scapularisin-3 and Scapularisin-6. Deduced amino acid sequences of prodefensins are 70 and 74 amino acids for Scapularisin-3 and Scapularisin-6, respectively. After cleavage, both mature proteins contain 38 amino acid residues (mature peptides). Within the α -core motif residues Thr10 and Ser13 in Scapularisin-3 are substituted with Ala10 and Arg13 in Scapularisin-6, enclosed in rectangles, and the six conserved cysteine residues are indicated by stars (♦ [57], ♦♦ [5]). (B) Tertiary structure of defensin peptides. Panel B displays the NMR defensin structure from *A. gambiae* (DEF-AAA; PDB: 2NY8) and the two predicted tertiary structures of Scapularisin-3 (GenBank: EEC13914) and Scapularisin-6 (GenBank: EEC08935). The tertiary structures depict the conserved disulphide bridges (roman numerals), loops, β -sheets, and the α -helix. All structures are coloured from the N-terminus (blue) to the C-terminus (red). Below are the respective electrostatic potentials for each structure in 180° turns (blue = positive; red = negative; white = neutral). (C) The protein backbone alignment in Panel C depicts each structure colour coded as indicated.

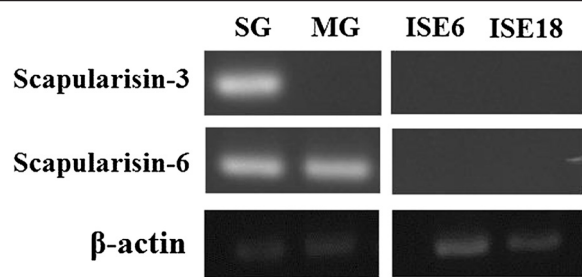


Figure 4 Expression of Scapularisin-3 and Scapularisin-6 in tick tissues and cell lines. The expression pattern determined by PCR and visualised by agarose gel electrophoresis of Scapularisin-3 and Scapularisin-6 in *I. scapularis* salivary glands (SG), midgut (MG) and embryo-derived tick cell lines ISE6 and ISE18 is shown. Both defensins were expressed in SG. Scapularisin-6 was expressed in MG and neither of the two defensins was expressed by embryo-derived tick cells (ISE6 and ISE18) *in vitro*. (β -actin was used as a positive control).

activity of the γ -core of Scapularisin-20. The activity of mature peptides compared to the activity of γ -core may differ. The fact that Scapularisin-6 showed specific antibacterial activity against *L. grayi* gains relevance considering that a growing number of *Listeria* strains resistant to antimicrobial compounds and particularly to antibiotics have been reported [28,50]. Thus, the identification of anti-listerial agents has become extremely important [51].

Remarkably, Scapularisin-3 and Scapularisin-6 exhibit high antifungal activities (with very low MIC of $\sim 1 \mu\text{M}$) against highly destructive plant pathogenic fungi, i.e., *Fusarium* spp. The ascomycete *F. graminearum* has major economic impact in the agriculture industry [52]. In general, the control of *Fusarium* spp has become challenging; for example azole, one of the available fungicides, is only moderately effective against *F. graminearum* [53]. Thus, new control methods are needed. Some results suggest that defensins in general are effective against *Fusarium* spp. The expression of radish defensin in transgenic wheat confers resistance to *F. graminearum* [54]. In addition, defensins isolated from the venom of the snake *Bothrops jararaca* [55], from the plant *Nicotiana glauca* [56] and from mice [57] have been shown to be effective against some species of *Fusarium*. In our study, the IC₅₀ against *F. culmorum* and *F. graminearum* of both Scapularisin-3 and Scapularisin-6 are considerably low ($1 \mu\text{M}$). When compared to Scapularisin-6, Scapularisin-3 caused growth inhibition of fungi at a lower concentration. However, our results suggest that Scapularisin-3 is more potent than Scapularisin-6 as antifungal agent. Scapularisin-3 has many substitutions to Arg when compared to Scapularisin-6. A recent study found that cationic residues (such as Arg) within the α -core and γ -core motifs found in plant defensins are important in antifungal activity against *F. graminearum* [58]. Further studies should be carried out in order to clarify the mechanistic actions of *I. scapularis*

defensins against fungi of the genus *Fusarium* and their potential for plant protection [59].

Here we demonstrated that Scapularisin-3 and Scapularisin-6 are transcribed differentially in *I. scapularis* salivary glands and midgut. This is in agreement with the expression patterns of defensins identified recently in *I. ricinus*, some of which seem to be tissue-specific and others ubiquitous [8]. Scapularisin-6 was previously shown to be expressed in the midgut, haemocytes, and fat-body of *I. scapularis* [60]. Our results confirm that it is expressed in the midgut and in addition we provide a new site of expression, the salivary glands. Hynes and colleagues mentioned that another gene expressed in the salivary glands from *I. scapularis* (AAV80792) has one leucine "L" at position 17 while Scapularisin-6 has one phenylalanine "F" and they speculated that "L" and not "F" was the salivary gland isoform. Our results show that isoform "F" is expressed in all the organs.

In contrast to the novel defensins from *I. ricinus* that were all constitutively expressed in the *I. ricinus* embryo-derived cell line IRE/CTVM19, except for DefMT5 [8], expression of the two *I. scapularis* defensins Scapularisin-3 and Scapularisin-6 was not detected in either of the embryo-derived tick cell lines ISE6 and ISE18. Although tick cell lines are generally quite heterogeneous, having been derived from multiple tissue types [39,40], they do not necessarily represent cells from the full complement of tick organs present in the starting material. ISE6 and ISE18 are relatively homogeneous lines compared to the *I. ricinus* line IRE/CTVM19 (authors' unpublished observations). It may be that the cell types expressing Scapularisin-3 and Scapularisin-6 in whole tick salivary glands and midgut are not present in the *I. scapularis* cell lines, while some of the cell types expressing the *I. ricinus* defensins are present in the more heterogeneous cell line derived from the latter species. Moreover, the pattern of defensin expression may differ between cells of tick embryos that have never experienced bacterial challenge or even a blood-meal, or partially-fed adult female ticks removed from a vertebrate host. Further studies involving *in vitro* microbial challenge of these and additional *I. scapularis* and *I. ricinus* cell lines [40] are needed to fully elucidate the expression patterns of defensins in embryo-derived cells.

Conclusion

Two defensins from *I. scapularis* were functionally characterised and they showed activity against fungi and the Gram + bacterium *L. grayi*. Both Scapularisin-3 and Scapularisin-6 were highly effective against *F. graminearum* and *F. culmorum* suggesting their promise for plant protection purposes. In addition, Scapularisin-6 has activity against the bacterium *L. grayi*. Our results confirm the antimicrobial activity of tick defensins and the potential of these defensins to be used as drugs against important agricultural and medical pathogens.

Abbreviations

AMP: Antimicrobial peptide; aa: Amino acid(s); kDa: Kilodalton(s); MIC: Minimum inhibitory concentration; IC₅₀: Half maximal inhibitory concentration.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MT designed the study, performed the functional analysis of synthetic peptides, interpreted the results, performed *in silico* analysis, wrote the manuscript and contributed to editing of the manuscript. ACC contributed to the overall design, supervision of the study to drafting the manuscript and editing of the manuscript. JJV performed tertiary structure predictions, refinement and analyses. TC designed primers and obtained the sequences. ROMR contributed to designing the molecular analyses, analysis of results and editing of the manuscript. MS performed RNA extraction, cDNA synthesis and PCR for the expression analysis. RŠ provided cDNA samples from tick organs. LBS provided tick cell lines and corrected English in the manuscript. ZF contributed to designing the molecular analyses. AV contributed to resource supply. LG contributed to design of the study and resource supply. MR designed, supervised the study and wrote the manuscript. All authors critically revised the manuscript and have given final approval of the version to be published.

Acknowledgments

We are grateful to Ulrike Munderloh, University of Minnesota, and the Tick Cell Biobank, The Pirbright Institute for providing ISE6 and ISE18 cell lines and Martin Palus for maintaining them. Miray Tonk is a Marie Curie Early Stage Researcher supported by the POSTICK ITN (Post-graduate training network for capacity building to control ticks and tick-borne diseases) within the FP7- PEOPLE – ITN programme (EU Grant No. 238511). This project was partially supported by the Grant Agency of the Czech Republic (GACR P302/11/1901) and with institutional support RVO: 60077344 from Biology Centre, Institute of Parasitology as well as grant ANTIGONE (EU-7FP; 278976). James J. Valdés was sponsored by project CZ.1.07/2.3.00/30.0032, co-financed by the European Social Fund and the state budget of the Czech Republic. Radek Šíma was supported by the Grant 13-12816P (GA CR). Mohammad Rahnamaeian and Andreas Vilcinskis acknowledge the Ministry for Science and Art of the State of Hesse (Germany) for funding the LOEWE Center of Insect Biotechnology and Bioresources. Zdeněk Franta was supported by Alexander von Humboldt Research Fellowship for Postdoctoral Researchers.

Author details

¹Biology Centre of the AS CR, Institute of Parasitology, Branišovská 31, 37005 České Budějovice, Czech Republic. ²Faculty of Science, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic. ³SaBio. Instituto de Investigación en Recursos Cinegéticos IREC, CSIC-UCLM-JCCM, Ronda de Toledo s/n, 13005 Ciudad Real, Spain. ⁴Center for Infection and Immunity of Lille (CIIL), INSERM U1019 – CNRS UMR 8204, Université Lille Nord de France, Institut Pasteur de Lille, Lille, France. ⁵The Pirbright Institute, Ash Road, Pirbright, Surrey GU24 0NF, UK. ⁶Fraunhofer Institute for Molecular Biology and Applied Ecology, Department of Bioresources, Winchester Strasse, D-35394 Giessen, Germany. ⁷Institute for Phytopathology and Applied Zoology, Justus-Liebig-University of Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany.

Received: 19 May 2014 Accepted: 21 November 2014

Published online: 03 December 2014

References

- Hajdušek O, Šíma R, Ayllón N, Jalovecká M, Perner J, de la Fuente J, Kopacek P: Interaction of the tick immune system with transmitted pathogens. *Front Cell Infect Microbiol* 2013, **3**:26.
- Taylor D: Innate immunity in ticks: a review. *J Acarol Soc Japan* 2006, **15**:109–127.
- Kopáček P, Hajdušek O, Burešová V, Daffre S: Tick innate immunity. *Adv Exp Med Biol* 2010, **708**:137–162. Review.

- Nakajima Y, van Naters-Yasui AV, Taylor D, Yamakawa M: Antimicrobial peptide defensin is involved in midgut immunity of the soft tick, *Ornithodoros moubata*. *Insect Mol Biol* 2002, **11**:611–618.
- Wang Y, Zhu S: The defensin gene family expansion in the tick *Ixodes scapularis*. *Dev Comp Immunol* 2011, **35**:1128–1134.
- Ganz T, Lehrer RI: Defensins. *Curr Opin Immunol* 1994, **6**:584–589.
- Lai R, Lomas LO, Jonczy J, Turner PC, Ress HH: Two novel non-cationic defensin-like antimicrobial peptides from haemolymph of the female tick, *Amblyomma hebraeum*. *Biochem J* 2004, **379**:681–685.
- Tonk M, Cabezas-Cruz A, Valdés JJ, Rego ROM, Rudenko N, Golovchenko M, Bell-Sakyi L, de la Fuente J, Grubhoffer L: Identification and partial characterisation of new members of the *Ixodes ricinus* defensin family. *Gene* 2014, **540**:146–152.
- Bulet P, Stöcklin R, Menin L: Anti-microbial peptides: from invertebrates to vertebrates. *Immunol Rev* 2004, **198**:169–184.
- Kocan KM, de la Fuente J, Manzano-Roman R, Naranjo V, Hynes WL, Sonenshine DE: Silencing expression of the defensin, varisin, in male *Dermacentor variabilis* by RNA interference results in reduced *Anaplasma marginale* infections. *Exp Appl Acarol* 2008, **46**:17–28.
- Pasupuleti M, Schmidtchen A, Malmsten M: Antimicrobial peptides: key components of the innate immune system. *Crit Rev Biotechnol* 2012, **32**:143–171.
- Bajard S, Rosso L, Fardel G, Flandrois JP: The particular behaviour of *Listeria monocytogenes* under sub-optimal conditions. *Int J Food Microbiol* 1996, **29**:201–211.
- Farber JM, Peterkin PI: *Listeria monocytogenes*, a food-borne pathogen. *Microbiol Rev* 1991, **55**:476–511.
- Hage E, Mpmugo O, Ohai C, Sapkota S, Swift C, Wooldridge D, Amar CFL: Identification of six *Listeria* species by real-time PCR assay. *Lett Appl Microbiol* 2014, **58**(6):535–540.
- Garrity GM, Bell JA, Lilburn TG: *Taxonomic Outline of the Prokaryotes*. Bergey's Manual of Systematic Bacteriology. 2. New York: Springer; 2004.
- Liu D: Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol* 2006, **55**:645–659.
- Graves LM, Helsel LO, Steigerwalt AG, Morey RE, Daneshvar MI, Roof SE, Orsi RH, Fortes ED: *Listeria marthii* sp. nov., isolated from the natural environment, Finger Lakes National Forest. *Int J Syst Evol Microbiol* 2010, **60**:1280–1288.
- Leclercq A, Clermont D, Bizet C, Grimont PAD, Le Flèche-Matéos A, Roche SM, Buchrieser C, Cadet-Daniel V: *Listeria rocourtiae* sp. nov. *Int J Syst Evol Microbiol* 2010, **60**:2210–2214.
- Bertsch D, Rau J, Eugster MR, Haug MC, Lawson PA, Lacroix C, Meile L: *Listeria fleischmannii* sp. nov., isolated from cheese. *Int J Syst Evol Microbiol* 2013, **63**:526–532.
- Lang HE, Neuhaus K, Scherer S: *Listeria weihenstephanensis* sp. nov., isolated from the water plant *Lemna trisulca* taken from a freshwater pond. *Int J Syst Evol Microbiol* 2013, **63**:641–647.
- Cummins AJ, Fielding AK, McLauchlin J: *Listeria ivanovii* infection in a patient with AIDS. *J Infect* 1994, **28**:89–91.
- Lessing MPA, Curtis GDW, Bowler ICJ: *Listeria ivanovii* infection. *J Infect* 1994, **29**:230–231.
- Guillet C, Join-Lambert O, Le Monnier A, Leclercq A, Mechali F, Mamzer-Bruneel MF, Bielecka MK, Scotti M: Human listeriosis caused by *Listeria ivanovii*. *Emerg Infect Dis* 2010, **16**:136–138.
- Rapose A, Lick SD, Ismail N: *Listeria grayi* bacteremia in a heart transplant recipient. *Transpl Infect Dis* 2008, **10**:434–436.
- Rocourt J, Hof H, Schrettenbrunner A, Malinverni R, Bille J: Acute purulent *Listeria seeligeri* meningitis in an immunocompetent adult. *Schweiz Med Wochenschr* 1986, **116**:248–251.
- Perrin M, Bemer M, Delamare C: Fatal case of *Listeria innocua* bacteremia. *J Clin Microbiol* 2003, **41**:5308–5309.
- Walker JK, Morgan JH, McLauchlin J, Grant KA, Shallcross JA: *Listeria innocua* isolated from a case of ovine meningoencephalitis. *Vet Microbiol* 1994, **42**:245–253.
- Salimnia H, Patel D, Lephart PR, Fairfax MR, Chandrasekar PH: *Listeria grayi*: vancomycin-resistant, gram-positive rod causing bacteremia in a stem cell transplant recipient. *Transp Infect Dis* 2010, **12**:526–528.
- Lucas S, Baştaş K, Budak H: Exploring the interaction between small RNAs and R genes during *Brachypodium* response to *Fusarium culmorum* infection. *Gene* 2014, **536**:254–264.
- Scherm B, Virgilio BS, Francesca S, Giovanna P, Giovanna D, Matias P, Quirico M: *Fusarium culmorum*: causal agent of foot and root rot and head blight on wheat. *Molec Plant Pathol* 2013, **14**:323–341.

31. Bormann J, Boenisch MJ, Brückner E, Firat D, Schäfer W: **The adenyl cyclase plays a regulatory role in the morphogenetic switch from vegetative to pathogenic lifestyle of *Fusarium graminearum* on wheat.** *PLoS ONE* 2014, **9**:e1135.
32. Pagnussatt FA, Del Ponte EM, Garda-Bufferon J, Badiale-Furlong E: **Inhibition of *Fusarium graminearum* growth and mycotoxin production by phenolic extract from *Spirulina* sp.** *Pestic Biochem Physiol* 2014, **108**:21–26.
33. Ward TJ, Clear RM, Rooney AP, O'Donnell K, Gaba D, Patrick S, Starkey DE, Gilbert J, Geiser DM, Nowicki TW: **An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America.** *Fungal Genet Biol* 2008, **45**:473–484.
34. Rahnamaeian M, Langen G, Imani J, Khalifa W, Altincicek B, von Wettstein D: **Insect peptide metchnikowin confers on barley a selective capacity for resistance to fungal ascomycetes pathogens.** *J Exp Bot* 2009, **60**:4105–4114.
35. Miedaner T, Reinbrecht C, Schilling AG: **Association among aggressiveness, fungal colonization, and mycotoxin production of 26 isolates of *Fusarium graminearum* in winter rye head blight.** *Zeitsch Pflanzenkrankh Pflanzenschutz* 2000, **107**:124–134.
36. Rahnamaeian M, Vilcinskis A: **Defense gene expression is potentiated in transgenic barley expressing antifungal peptide metchnikowin throughout powdery mildew challenge.** *J Plant Res* 2012, **125**:115–124.
37. Fogaça AC, Lorenzini DM, Kaku LM, Esteves E, Bulet P, Daffre S: **Cysteine-rich antimicrobial peptides of the cattle tick *Boophilus microplus*: isolation, structural characterization and tissue expression profile.** *Dev Comp Immunol* 2004, **28**:191–200.
38. Kurti TJ, Munderloh UG, Andreadis TG, Magnarelli LA, Mather TN: **Tick cell culture isolation of an intracellular prokaryote from the tick *Ixodes scapularis*.** *J Invert Pathol* 1996, **67**:318–321.
39. Munderloh UG, Liu Y, Wang M, Chen C, Kurti TJ: **Establishment, maintenance and description of cell lines from the tick *Ixodes scapularis*.** *J Parasitol* 1994, **80**:533–543.
40. Bell-Sakyi L, Zweggarth E, Blouin EF, Gould EA, Jongejan F: **Tick cell lines: tools for tick and tick-borne disease research.** *Trends Parasitol* 2007, **23**:450–457.
41. Kelley LA, Sternberg MJE: **Protein structure prediction on the Web: a case study using the Phyre server.** *Nat Protocols* 2009, **4**:363–371.
42. Li X, Jacobson MP, Zhu K, Zhao S, Friesner RA: **Assignment of polar states for protein amino acid residues using an interaction cluster decomposition algorithm and its application to high resolution protein structure modeling.** *Proteins Struct Funct Bioinformatics* 2007, **66**:824–837.
43. Landon C, Barbault F, Legrain M, Guenneugues M, Vovelle F: **Rational design of peptides active against the gram positive bacteria *Staphylococcus aureus*.** *Proteins* 2008, **72**:229–239.
44. Chrudimská T, Chrudimský T, Golovchenko M, Rudenko N, Grubhoffer L: **New defensins from hard and soft ticks: similarities, differences, and phylogenetic analyses.** *Vet Parasitol* 2010, **167**:298–303.
45. Saito Y, Konnai S, Yamada S, Imamura S, Nishikado H, Ito T, Onuma M, Ohashi K: **Identification and characterisation of antimicrobial peptide, defensin, in the taiga tick, *Ixodes persulcatus*.** *Insect Mol Biol* 2009, **18**:531–539.
46. Yu D, Sheng Z, Xu X, Li J, Yang H, Liu Z, Rees HH, Lai R: **A novel antimicrobial peptide from salivary glands of the hard tick, *Ixodes sinensis*.** *Peptides* 2006, **27**:31–35.
47. Lu X, Che Q, Lv Y, Wang M, Lu Z, Feng F, Liu J, Yu H: **A novel defensin-like peptide from salivary glands of the hard tick, *Haemaphysalis longicornis*.** *Protein Sci* 2010, **19**:392–397.
48. Nakajima Y, van Naters-Yasui AV, Taylor D, Yamakawa M: **Two isoforms of a member of the arthropod defensin family from the soft tick, *Ornithodoros moubata* (Acari: Argasidae).** *Insect Biochem Mol Biol* 2001, **31**:747–751.
49. Tsuji N, Battsetseg B, Boldbaatar D, Miyoshi T, Xuan X, Oliver JH Jr, Fujisaki K: **Babesial vector tick defensin against *Babesia* sp. parasites.** *Infect Immun* 2007, **75**:3633–3640.
50. Schwaiger K, Schmied EM, Bauer J: **Comparative analysis on antibiotic resistance characteristics of *Listeria* spp. and *Enterococcus* spp. isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany.** *Zoonoses Pub Hlth* 2009, **57**:171–180.
51. Mukherjee K, Abu Mraheil M, Silva S, Müller D, Cemic F, Hemberger J, Hain T, Vilcinskis A, Chakraborty T: **Anti-*Listeria* activities of *Galleria mellonella* hemolymph proteins.** *Appl Env Microbiol* 2011, **77**:4237–4240.
52. Nielsen LK, Cook DJ, Edwards SG, Ray RV: **The prevalence and impact of *Fusarium* head blight pathogens and mycotoxins on malting barley quality in UK.** *Int J Food Microbiol* 2014, **28**(179C):38–49.
53. Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD: **The Top 10 fungal pathogens in molecular plant pathology.** *Mol Plant Pathol* 2012, **13**:414–430.
54. Li Z, Zhou M, Zhang Z, Ren L, Du L, Zhang B, Xu H, Xin Z: **Expression of a radish defensin in transgenic wheat confers increased resistance to *Fusarium graminearum* and *Rhizoctonia cerealis*.** *Funct Integr Genomics* 2011, **11**:63–70.
55. Gomes VM, Carvalho AO, Da Cunha M, Keller MN, Bloch C Jr, Deolindo P, Alves EW: **Purification and characterization of a novel peptide with antifungal activity from *Bothrops jararaca* venom.** *Toxicon* 2005, **45**:817–827.
56. Dracatos PM, van der Weerden NL, Carroll KT, Johnson ED, Plummer KM, Anderson MA: **Inhibition of cereal rust fungi by both class I and II defensins derived from the flowers of *Nicotiana glauca*.** *Mol Plant Pathol* 2014, **15**:67–79.
57. Kolar SS, Baidouri H, Hanlon S, McDermott AM: **Protective role of murine β -defensins 3 and 4 and cathelin-related antimicrobial peptide in *Fusarium solani* keratitis.** *Infect Immun* 2013, **81**:2669–2677.
58. Sagaram US, Pandurangi R, Kaur J, Smith TJ, Shah DM: **Structure-activity determinants in antifungal plant defensins MsDef1 and MtDef4 with different modes of action against *Fusarium graminearum*.** *PLoS ONE* 2011, **6**:e18550.
59. Rahnamaeian M: **Antimicrobial peptides: modes of mechanism, modulation of defense responses.** *Plant Signal Behav* 2011, **6**(9):1325–1332.
60. Hynes WL, Ceraul SM, Todd SM, Seguin KC, Sonenshine DE: **A defensin-like gene expressed in the black-legged tick, *Ixodes scapularis*.** *Med Vet Entomol* 2005, **19**(4):339–344.

doi:10.1186/s13071-014-0554-y

Cite this article as: Tonk et al.: Defensins from the tick *Ixodes scapularis* are effective against phytopathogenic fungi and the human bacterial pathogen *Listeria grayi*. *Parasites & Vectors* 2014 **7**:554.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

