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Expressed sequence tags in venomous tissue of *Scorpaena plumieri* (Scorpaeniformes: Scorpaenidae)

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Species of the family Scorpaenidae are responsible for accidents and sporadic casualties by the shore they inhabit. The species *Scorpaena plumieri* from this family populate the Northeastern and Eastern coast of Brazil causing human envenomation characterized by local and systemic symptoms. In experimental animals the venom induces cardiotoxic, hypotensive, and airway respiratory effects. As first step to identify the venom components we isolated gland mRNA to produce a cDNA library from the fish gland. This report describes the partial sequencing of 356 gland transcripts from *S. plumieri.* BLAST analysis of transcripts showed that 30% were unknown sequences, 17% hypothetical proteins, 17% related to metabolic enzymes, 14% belonged to signal transducing functions and the remaining groups (7-8%) composed by gene related with expressing proteins, regulatory proteins and structural proteins. A considerable number of these EST were not found in available databases suggesting the existence of new proteins and/or functions yet to be discovered. By screening the library with antibodies against a lectin fraction from *S. plumieri* venom we identified several clones whose DNA sequence showed similarities with lectins found in fish. *In silico* analysis of these clones confirm the identity of these molecules in the venom gland of *S. plumieri.*

Espécies da família Scorpaenidae são responsáveis por acidentes e mortes esporádicas ao longo da costa que habitam. A espécie *Scorpaena plumieri* desta família povoam a costa Leste e Nordeste do Brasil, causando envenenamento humano caracterizado por sintomas locais e sistêmicos. Em modelos experimentais animais a peçonha induz cardiotoxicidade, efeitos hipotensivos e alterações nas vias aéreas respiratórias. Como primeiro passo para identificar os componentes da peçonha foram isolados os mRNA das glândulas do peixe para produzir uma biblioteca de cDNAs. Esse artigo descreve o sequenciamento parcial de 356 transcritos das glândulas de *S. plumieri*. Análises em bancos de dados (BLAST) dos transcritos demonstraram que 30% eram sequências desconhecidas, 17% proteínas hipotéticas, 17% relacionadas às enzimas do metabolismo, 14% pertenciam a funções de transdução de sinais e os demais grupos (7-8%) formados por genes relacionados com a expressão de proteínas, proteínas regulatórias e estruturais. Um número considerável destes EST não foi encontrado em bases de dados disponíveis, sugerindo a existência de novas proteínas e/ou funções ainda a serem descobertas. Ao fazer um barrido da biblioteca com anticorpos produzidos contra uma fração das lectinas do veneno de *S. plumieri*, identificamos vários clones, cuja sequência de DNA mostram semelhanças com lectinas encontradas em peixes. A análise *in silico* destes clones confirmam a identidade destas moléculas na glândula de peçonha de *S. plumieri*.

Key words: cDNAs, EST, Glands, Lectin, Scorpionfish, Toxins.

Introduction

The animal kingdom contains more than 100,000 species that synthesize venoms used by the host to protect against predators or to subdue a victim before ingestion (Mebs, 2002). Venomous fish synthesize toxins in specialized glands-like compartments containing spines situated on the chest, dorsal, gill or caudal areas and surrounded by a tegumental sheet (Russel, 1965). Scorpionfish encompasses three groups of venomous fish (*Pterois*, *Scorpaena*, and *Synanceia*) with ubiquitous distribution in tropical and temperate seas (Halstead, 1980; Williamson *et al*., 1996). The venom contains a myriad of molecules acting on various exogenous substrates, *i.e.*, ion channel, chemical receptors or molecular structures in

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target organisms. Some small venom components are already known such as acetylcholine, cathecolamines, and histamine, but mostly unknown proteinaceous molecules are also present (Church & Hodgson, 2002; Figueiredo *et al*., 2009).

Scorpaena plumieri known in Brazil as aniquim, mamangá, or moréia-atí is abundant along the Brazilian coast (Menezes & Figueiredo, 1980; Carvalho-Filho, 1999) and is widely found in shallow-water bottom dwellers, bays, along sandy beaches, rocky coastlines or coral reefs. Specimens have a bizarre appearance, habits of concealing themselves in crevices, among debris, under rocks that together with their protective coloration which blends them almost perfectly into their surrounding environment, makes them difficult to see, predisposing to accidents (Russel, 1965; Schaeffer *et al*., 1971).

This species is held responsible for accidents that cause injuries to humans, especially, fishermen and professional swimmers. Envenomation is mostly non-life-threatening to humans and is characterized by local edema and erythema. Systemic symptoms such as; cardiotoxic and vasorelaxant effects may be severe, resulting in drastic drop of blood pressure (Carrijo *et al*., 2005; Boletini-Santos *et al*., 2008; Haddad *et al*., 2003; Loyo *et al*., 2008). During envenomation there is an increase of bronchi and epithelial permeability similar to that observed during erythema or hemorrhage, suggesting a change of cell matrix interactions (Theakston & Kamiguti, 2002).

A cytolytic toxin (Sp-CTx) has been purified from the venom of *S. plumieri* by hydrophobic interaction and anion exchange chromatographies with estimated molecular mass of 150 kDa. Further, the protein is dimeric comprising subunits of approximately 75 kDa each, similar to what has been described in other stonefish species (Gomes *et al*., 2013). Sp-CTx displays potent hemolytic activity on washed rabbit erythrocytes (EC_{50} 0.46 nM); its effect is antagonized by antivenom raised against stonefish venom - *Synanceia trachynis* (SFAV). Like *S. plumieri* whole venom (100 µg/mL), Sp-CTx (1-50 nM) causes a biphasic response on phenylephrine pre-contracted rat aortic rings, characterized by an endothelium- and dose-dependent relaxation phase followed by a contractile phase (Andrich *et al*., 2010).

However, local envenomation effects are also attributed to the presence of the β-lectin *plumieribetin* isolated from *S. plumieri* (De Santana Evangelista, 2009). The fully characterized protein (14.4 kDa) acts as α1β1 integrin inhibitor similar to monocot mannose-binding B-lectins and to pufflectin found in skin and intestine of Japanese pufferfish/*Fugu* fish (*Takifugu rubripes*) (Tsutsui *et al*., 2003).

Due to limited amounts available of these bioactive molecules and because of their instability, venomous fish have remained unexplored (Figueiredo *et al*., 2009). The lack of studies of the functional venom genes of *S. plumieri* moved us to generate a cDNA library to analyze by EST, the expressed components of the venom gland. Using this approach we expect to find novel genes, transcription profiling and a comparison with homologous genes found in related species.

Material and Methods

RNA Extraction

Wild specimens of *Scorpaena plumieri* were fished off the coast of Espírito Santo, Brazil and kept in captivity prior to gland dissection. The tissue was extracted from the dorsal and caudal venom glands of two male young adult exemplars and kept in liquid N_2 during initial grinding with a tissue grinder mill. The RNA was extracted using guanidinium thiocyanate-phenol-chloroform as described earlier (Chomczynski & Sacchi, 1987). Poly A RNA was obtained by chromatography of total RNA in oligo-(dT) cellulose. cDNA was synthesized starting with 0.5 µg of polyA RNA using the ZAP-cDNA synthesis kit (ZAP-cDNA Gigapack III gold cloning kit, GE). After size fractionation on a CL-2B gel filtration column the cDNA was precipitated, resuspended and ligated to the Uni-ZAP XR vector following the supplier protocol.

Library titration

The number of clones in the primary library was determined using the relation; pfu/mL = ^{1} pfu x 10^{3} µL/mL x dilution factor. The recombination efficiency was established by scoring white/blue colonies using *E. coli* XL1-blue cells, in the presence of 2.5 mM IPTG and 50 mg/mL X-gal in DMF. The insert size was determined by PCR of colonies, enzyme digestion of recovered plasmid DNA and 1% agarose gel electrophoresis. The amplified library was stored at -80°C in 7% DMSO.

DNA sequencing and analysis

cDNA clones randomly selected from the library were sequenced at the 5' end with the automatic sequencer 3.100 Genetic (Applied Biosystems) according to the protocol provided by the supplier BigDye™ Terminator Ready Reaction Mix (Sanger, 1977). The processed DNA sequences were analyzed using the NCBI databank (http://www.ncbi.nlm.nih.gov/blast) to search for similarities. Blast scores higher than 80 and E-values $\leq 10^{-10}$ were considered as significant (Thanh *et al*., 2011). Protein alignments analysis was done using the tools of Expasy, http://www.expasy.ch).

Library screening with gland venom antibodies fraction.

The antiserum against a lectin fraction was raised in a rabbit according to standard protocols at the animal house at Ezequiel Dias Foundation R & D Center (Belo Horizonte, Brazil).The library screening procedure adopted was as previously described with modifications (Ausubel, 1995). Briefly, the phagemid library was plated onto culture plates (15 cm ø) and grown during 3 h at 37°C before laying onto the plate a nitrocellulose membrane previously soaked with 10 mM IPTG, and the culture further incubated for 3 h at 37°C. The nitrocellulose filters were lysed with buffer: (50 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 150 mM NaCl, 1 µg/mL DNAase, 40 µg/mL lysozyme and 3% BSA). Then, the immunoscreening

buffer (TBS, 5% BSA and 3 mM NaN_3 (4x) containing antibody was added at a concentration of 10 µg/mL (1:1000) in PBS-T for 1 h at 37°C. After washings with the immunoscreening buffer, the phosphatase secondary antibody was added and incubated 1 h at 37°C (1:10000) and following several washings, the filter was developed with BCIP-NBT substrate. Brown colonies were isolated at the master plate after alignment to the membrane and their DNA sequenced.

Results

Total RNA obtained from fish gland exhibited a 280/260 ratio of 1.9. To verify its integrity an aliquot was electrophoresed on agarose-formaldehyde gel. The result in Fig. 1 shows the 28 and 18S rRNA bands plus a smear of RNA. Incubation of the RNA sample during 2 h at 37°C showed no changes in RNA distribution, thus confirming the stability of this preparation. cDNA was prepared using the cDNA synthesis kit provided by Agilent Thecnologies, following the supplier instructions (ZAP-cDNA Gigapack III Gold Cloning Kit, La Jolla, USA). Before ligation to bacteriophage arms the cDNA was sized separated on Sepharose CL-2B to select larger cDNA populations. The pooled cDNA was concentrated and ligated overnight with the Uni-ZAP vector and packaged with Gigapack III Gold packaging extract following the manufacturer instructions (Stratagene Products, La Jolla, USA). Colonies containing insert were selected by adding IPTG, X-gal to plates. The primary library totalized HH~1.85 x 10^5 pfu and the percentage of recombinants amounted to 90%. Following amplification the title increased to 2.5 X 10^9 pfu/mL and the recombinant ratio decreased to 65%. Mass excision of the library to release the phagemid used 1.85 X 10^7 pfu and a 100-fold excess of helper Ex-assist phage, as suggested by the supplier (ZAP-cDNA Gigapack III Gold Cloning Kit).

EST sequence and analysis

Four-hundred and seventy white colonies randomly selected were grown in liquid culture medium and the recovered plasmid DNA quantified by agarose electrophoresis and digested with *Eco*R1 to confirm the presence of insert (Fig. 2). After removal of the vector contamination by low-quality DNA sequences and ribosomal RNA sequences, 356 individual clones were selected and subjected to further analysis. The EST size were 0.2-0.5 kb (31.4%), 0.5-1.0 kb (39.4%) and 1.0-2.0 kb (29.2%). The purified DNA was sequenced using M13 primers and after sequence edition submitted to Blastn for analysis. The ESTs showing relationship with previously identified sequences were classified in categories using the function attributed to the original sequence. If an EST shared homology with more than one category in the data bank, the classification opted for the main putative function.

The EST distribution in Fig. 3 shows a large number of unknown sequences (n=107, 30%), followed by (n=61, 17%) hypothetical proteins (as designated in databanks) and similar amount of metabolic enzymes (n=61, 17%), next, proteins linked to signaling functions (n=49, 14%), and the remaining groups composed by gene expressing proteins, regulatory proteins and structural proteins (n=78, 7-8%).

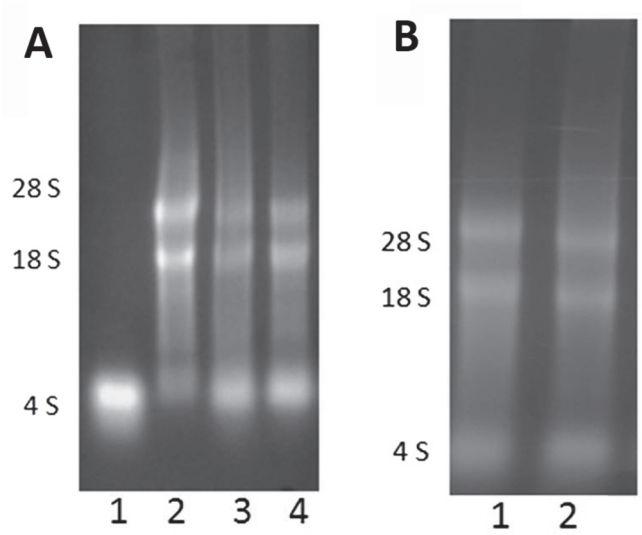

Fig. 1. Agarose- formaldehyde electrophoresis of RNA from *Scorpaena plumieri*. **A)** 1) 2 µg of *E. coli* tRNA; 2) 2 µg de rRNA de *Rattus norvegicus*; 3) and 4) 2 µg total RNA from *S. plumieri* spine gland. **B)** 1) 2 µg de total RNA from *S. plumieri*; 2) the same sample incubated 2 h a 37ºC before electrophoresis.

We selected 115 ESTs, whose identities strongly match blastn entries, and scoring ≥ 80. Table 1 lists the nucleotide putative match and GeneBank accession number for each entry. This list contains, 63 (55%) single copies and 52 ESTs (46%) corresponding to two or more copies identified in the library. One-hundred nineteen (33%) of these ESTs displayed a strong match (score > 401), meanwhile 57 of them (25%) had a score between 80 - 400, and the 92 remaining EST (31%) had a score below 80.

We next screened the library with antibodies against a venom lectin fraction from *S. plumieri* and identified several clones sharing homology with lectins from *Oplegnathus fasciatus*, *Dicentrarchus labrax*, *Maylandia zebra*, and *Oreochromis niloticus*. In this analysis we detected an ORF containing 267 residues sharing three cysteine residues featured by lectins and compatible in size with plumieribetin. The alignment of this lectin-like sequence is shown in Fig. 4. Some invariant amino acid residues ($Asp^{81}$and Asn^{125}) described at the top of the dome-shaped domain structure in all legume lectins sequenced so far (Asp^{207} and Asn^{253} in this sequence), is responsible for sugar specic recognition (Fig. 4).

Discussion

Due to the diversity of effects by the venom of *Scorpaena plumieri* and the lack of information about these venoms, we decided to study the molecular diversity of fish venom. To accomplish this goal, a cDNA library was generated and partial

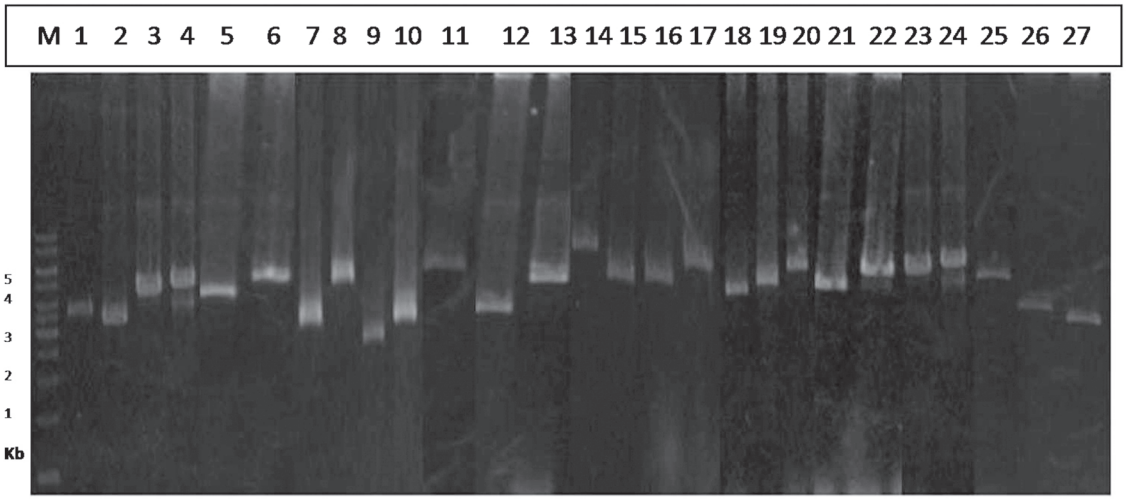

Fig. 2. Agarose gel electrophoresis of DNA isolated from clones. White colonies containing insert were grown and the plasmidial DNA isolated and digested with *Eco*RI enzyme. An aliquot from each clone (1-27) was electrophoresed on 1% Agarose gel and stained with ethidium bromide.

sequencing of the cDNAs performed. The expressed sequence tag (EST) approach provides a rapid and reliable method for gene discovery as well as a source of new annotations for analysis of known and unknown expressed transcripts.

As first step to characterize the library, we randomly sequenced 470 colonies containing inserts. After removal of ribosomal RNA sequences and low quality sequences, we generated three-hundred fifty-six ESTs, some of them sharing similarity with previously described sequences found in fish and others species. The majority of inserts had size range between 0.5 - 1 Kb (39%) and similar amounts (31%) of smaller size inserts (0.2-0.5 Kb) and larger inserts (1- 2 Kb) 29%. The edited sequences were submitted to Blastn and the resulting matches were classified in categories according to the function originally assigned in the databank. The distribution pie in Fig. 3 shows that 30% of EST corresponded to unknown sequences, followed by similar amounts (17%) of hypothetical proteins, metabolic related proteins, and signaling proteins. The large proportion of unknown functions argues for the presence of new non-catalogued proteins and or peptides whose role has not been yet defined. Interestingly, in a similar EST study of fish venom gland from *Thalassophryne nattereri* it was also described an expressive amount of unknown sequences (39%) suggesting the existence of yet unidentified proteins in both marine species (Magalhães *et al*., 2006). The presence of additional toxic sequences cannot be ruled out at this point since the EST sequencing project is under way.

Among matching sequences we identified a clone whose partial sequence aligned with *patoxin*-β subunit mRNA from *Pterois antennata* (score 1642) (Table 1). The isolate will be further investigated to establish if corresponds to the complete structure of this toxin, in which case it will be expressed in *E. coli* to study the gene product.

We also found a sequence matching a protein involved in cholesterol transport as; *apolipoprotein E* precursor and genes related to cell signaling as integrins. Ca^{2+} binding proteins including a sequence related to S-100 and another to *annexin 1A*, were detected. S-100 protein has been identified in exocrine glands and thought to play a role in secretion (Case *et al*., 1988). Together with annexin 1A it can form heterocomplexes due to increase in intracellular Ca^{2+}, which stimulates venom secretion. Therefore, the presence of these transcripts in *S. plumieri* library suggests that these genes might be involved in toxin secretion within the venomous apparatus.

The sequencing of EST showed transcripts that matched fish DNA sequences responsible for protecting proteins from

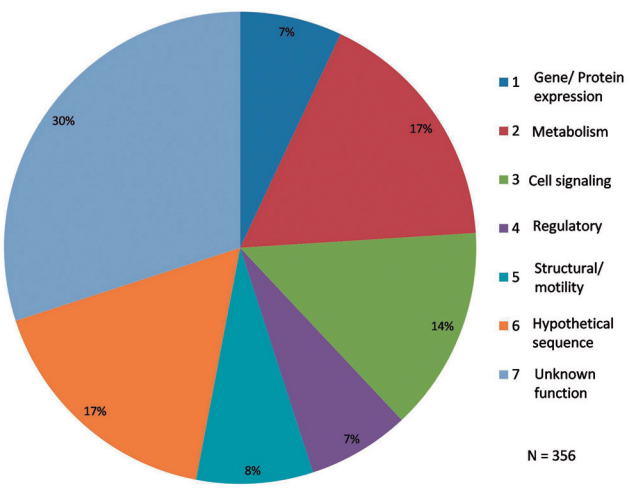

Fig. 3. The classification of EST from *Scorpaena plumieri* based on their putative fractions. Three-hundred fifty-six EST edited sequences were initially analyzed with Blast and Swiss protein databanks. The consensus sequence was attributed a function based on the strongest match.

Table 1. Representative list of EST matches with blastn from randomly sequenced clones from *Scorpaena plumieri*. ($\leq 10^{-10}$, score ≥ 80).

Group / HIT ID	**E-Value (score)**	**Entry identity**	**№ of ESTs**
Metabolism			
FJ629181.1	0.0 (1282)	Kinase I	1
BT083258.1	0.0 (1097)	p-21-activated protein kinase	1
XM_003446848.1	0.0 (905)	ADP/ATP translocase 2-like	2
EU036495.1	1e-179 (640)	Cytochrome b	7
FJ426129.1	2e-177 (632)	Chaperonin subunit 7	1
DQ900715.1	7e-149 (536)	Cytochrome c oxidase subunit II	3
XM_003459458.1	6e-119 (437)	N-acetylgalactosaminyltransferase-like	2
FJ826528.1	5e-70 (275)	Malate dehydrogenase	1
XM_003454129.1	2e-13 (86)	Protein tyrosine phosphatase type IVA	1
Structure/Motility			
JF317678.1	0.0 (1387)	β-actin	4
JN112540.1	0.0 (1225)	Keratin 8	1
AB196514.1	0.0 (1151)	Collagen, type 1, alpha 2	5
AF263276.1	0.0 (959)	Alpha tubulin	1
XM_003449910.1	0.0 (937)	T-complex protein 1 subunit alpha	1
AB603658.1	0.0 (926)	Collagen, type 1, alpha 3	1
AB196514.1	0.0 (911)	Collagen, type 1, alpha 1	3
AB490880.1	0.0 (699)	Ferritin heavy chain	1
BT082330.1	7e-136 (494)	Peripheral myelin	1
DQ364242.1	3e-125 (459)	Keratin 1	2
XM_004070275.1	9e-100 (374)	Intermediate filament protein ON3	1
BT083320.1	3e-83 (318)	MANBAL	1
XM_003452967.1	8e-43 (185)	Ubiquitin-conjugating enzyme E2	1
XM_002665878.3	3e-23 (120)	Procollagen, type V, alpha 1	1
AY787209.2	8e-20 (107)	Cartilage-specific S100-like protein	1
Cell signaling/Cell communication			
BT028386	0.0 (1325)	Guanine nucleotide binding protein	3
AB326303.1	0.0 (1129)	Elongation factor 1-alpha	5
XM_003442398.1	0.0 (996)	Coronin-1A-like	1
XM_003449701.1	0.0 (990)	Arfaptin-1-like	1
XM_003449090.1	0.0 (900)	Angiopoietin	1
FJ455761.1	0.0 (776)	QM-like protein	1
GU988616.1	7e-161 (577)	Annexin A11	1
XM_003451608.1	7e-161 (577)	MYC binding protein 2	1
AB618145.1	1e-96 (449)	Fish-egg lectin	24
FJ800037.1	9e-109 (403)	Apolipoprotein E	1
AB201746.1	3e-106 (394)	Lily-type lectin	1
XM_003441063.1	4e-100 (374)	PDZ and LIM domain protein 1-like	1
GU060308.1	1e-74 (289)	S100-like calcium binding protein	1
HQ441029.1	2e-64 (255)	Poly A binding protein, citoplasmatic 1b	1
XM_003445768.1	6e-62 (248)	C3a anaphylatoxin chemotactic receptor	1
XR_134816.1	4e-52 (215)	Fibronectin-like	1
XM_003449138.1	1e-27 (200)	von Willebrand factor A domain	4
X81969.1	8e-45 (190)	D1c dopamine receptor	1
XM_003449453.1	1e-41 (179)	SUN domain protein	1
BT044760.1	6e-27 (131)	Ras-related protein Rab-10	1
FJ826549.1	5e-26 (129)	C-type lectin 7	2
FJ826556.1	4e-21 (111)	C-type lectin 14	1
AB084425.1	1e-20 (109)	Solute carrier family 26	1
XM_003438149.1	2e-18 (104)	Calcyclin-binding protein	1
HQ441075.1	2e-11 (80.6)	Cofilin-2	1
Regulatory			
HQ646108.1	0.0 (1308)	Heat shock cognate 70	2
XM_003451520.1	0.0 (1201)	Thrombospondin-4-B-like	1
FJ644278.1	0.0 (1090)	Heat shock protein 90	2
AY647431.1	1e-142 (516)	IRF-1	1
BT046051.1	3e-31 (145)	Profilin-2	1
XM_003455475.1	4e-30 (141)	Thymosin beta-12-like	2
XM_003437992.1	2e-26 (129)	Integrin alpha-6-like	1
AJ556548.1	2e-16 (96.9)	TRAF4 protein	1
HQ447060.1	2e-12 (84.2)	Haplotype 2 AFGP/TLP	1
Others			
AB623221.1	0.0 (1642)	Patoxin subunit b	1

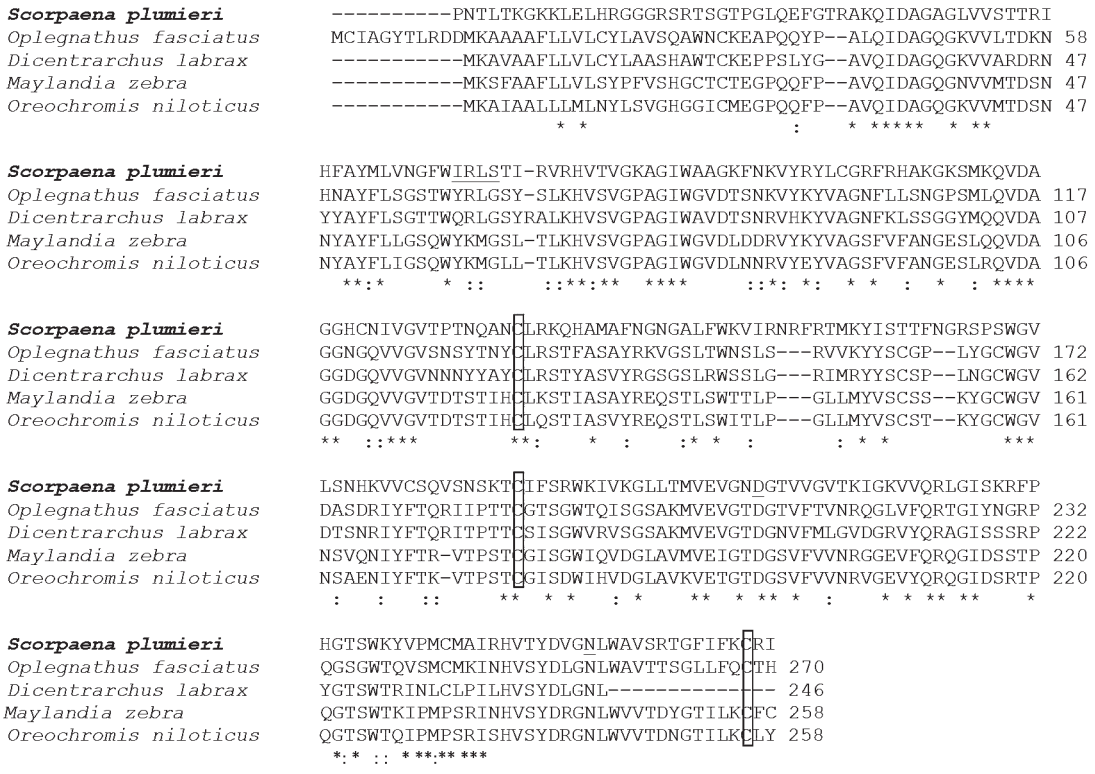

Fig. 4. Sequence alignment of putative lectin from *Scorpaena plumieri*. Alignment of a lectin-like EST *in silico* translated sequence from *S. plumieri* (ClustalW2 EBI) with fish-egg lectin from *Oplegnathus fasciatus* (BAL618145), *Dicentrarchus labrax* (CBK52298), *Maylandia zebra* (XP_004574029), and *Oreochromis niloticus* (XP003443389). The recombinant clone was isolated with antibody fraction derived from *S. plumieri* venom. * identifies and identical residue; **:** identifies a conserved residue. Underlined residues represent invariable sites, underlined IRLS = N-acetylation site.

degradation and necessary during post-translational processing (Hsp70, Hsp90, and one chaperonin subunit). Heat shock proteins and other chaperonins are a subset of ubiquitous proteins that direct the folding and assembly of cellular proteins (Welch, 1991). Recent reports show that Hsps levels increase in fish tissues in response to a variety of environmental and biological stressors (Iwama *et al*., 1999). Interestingly, we also found genes involved in homeostasis, such as fibronectin-like, angiopoietin, and von Willebrand factor, as well as defense genes involved in the immune response, such as the immunoglobulin heavy chain, thymosin, coronin-1A-like and IRF-1, an a transcription factor that plays a critical role in antiviral defense and immune response (Shi *et al*., 2010). ESTs showing homology with genes related to innate immunity, such as ferritin, thrombospondin and fish egg lectins were also found in the library.

Fish egg lectins were highly abundant in venom glands of *S. plumieri* and few have been already reported in marine animals, particularly fish. A chemoattractant lectin from the dorsal spines of the redfin velvetfish, *Hypodytes rubripinnis* (= *Paracentropogon rubripinnis*) was isolated and reported its chemoattractant activity. This glycoprotein induced agglutination of rabbit erythrocytes and was effectively inhibited by D-mannose (Shinohara *et al*., 2010).

We next screened the library with antibodies against a venom lectin fraction from *S. plumieri* and identified several clones sharing homology with lectins from *Oplegnathus fasciatus*, *Dicentrarchus labrax*, *Maylandia zebra*, and *Oreochromis niloticus* (Fig. 4). Lectins are agglutinating proteins that recognize specific glycoproteins and glycoconjugates at the cell surface.

In a recent study a lectin-like molecule called plumieribetin was described in venomous glands of *S. plumieri* that inhibits integrin binding to collagen IV from the basement membrane. The inhibition contributes to the local and systemic effect of envenomation by scorpionfish (De Santana Evangelista, 2009). Plumieribetin is a homotetrameric protein displaying high content of antiparallel B-strands, similar to the mannose-binding monocotiledons-B-lectins. Plumieribetin lacks N-linked glycoconjugates and common O-glycan motifs found in plant B-lectins, these modifications are necessary for binding of plant lectins to integrins, therefore, it was proposed that plumieribetin binds directly to integrins. The fully characterized protein (14.4 kDa) acts as $\alpha 1\beta 1$ integrin inhibitor similar to monocot mannose-binding B-lectins and to pufflectin found in skin and intestine of Japanese pufferfish/*Fugu* fish (*Takifugu rubripes*) (Tsutsui *et al*., 2003).

Analysis of the lectin-like sequence in the library identified an ORF with 267 residues displaying three cysteine residues also featured by lectins and compatible in size with plumieribetin. The alignment of this lectin-like sequence is shown in Fig. 4. Some invariant amino acid residues ($Asp^{81}$and Asn^{125}) described at the top of the dome-shaped domain structure in all legume lectins sequenced so far (Asp^{207} and Asn^{253} in this sequence), are responsible for sugar specic recognition (Fig. 4).

In silico analysis (Expasy, BLAST, UniProt) of the consensus sequence suggests the presence of the β-propeller structure found also in tachylectin-2, a five-blade propeller domain described in *Tachypleus tridentatus* crab (Medzhitov & Janeway, 1997). Inspection of potentially modified sites shows absence of glycosylation sites, one N-acetylation site and six lysine glycation sites. Prediction of secondary structures Expasy, SOPMA) suggests the prevalence of tandem β-sheets (41%), followed by random coil (39%) and α-helix (9%). The presence of tandem β-sheets has been frequently reported in mannose binding plant lectins (Barre *et al*., 2001). However, it is possible that fish-toxin lectins may contribute with the local and systemic effects observed on envenomation such as severe pain, swelling and fever (Shinomara *et al*., 2010). Future experiments must address the expression of this protein to evaluate its biological activity.

Acknowledgments

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