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FIRST REPORT OF LIVIDIN AND SPINULOSAIN PEPTIDES FROM THE SKIN SECRETION OF AN INDIAN FROG

SHORT COMMUNICATION

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Here, we report two novel peptides identified from the skin secretion, having homologies to Lividin and Spinulosain, of an endemic frog, *Hydrophylax bahuvistara,* of Western Ghats. This is the first report of these peptides from Indian frogs and first identification of Lividin from the *Hydrophylax* genus. Both peptides exhibited weak antimicrobial activity but very low haemolytic activity. The problems of naming amphibian host defense peptides (HDPs) are also discussed.

Keywords: Lividin – Spinulosain – Hylarana – nomenclature – antimicrobial

Development of Host Defense peptide (HDP) libraries from the skin secretion of amphibians is currently gaining momentum not only because of their characteristic feature to disrupt biological membranes [6] but also their proposed therapeutic potentials. HDPs are a cocktail of compounds, especially peptides, having broad spectrum biological activities which act as a first line of defense and secreted upon stress or injury [2]. In this study, two novel peptides having homologies to Lividin and Spinulosain were identified from the skin secretion of *Hydrophylax bahuvistara,* an endemic frog species of Western Ghats, India. Skin secretion harvesting and primary structure elucidation of the peptides were done as per standard procedures [1]. The peptide sequence obtained was subjected to homology searches using BLAST (NCBI) and their physicochemical properties were computed (http://expasy.org/tools/protparam.html). Helical wheel of the peptides were plotted (Don Armstrong and Raphael Zidovetzki. Version: Id: wheel. pl, v 1.4 2009-10-20 21:23:36 don ExpIdentified) followed by chemical synthesis of peptides. Minimal inhibitory concentrations (MICs) were determined [5] against some bacterial strains (Table 1B) and were further confirmed by resazurin microtiter assay (REMA). Hemolytic activities of these peptides on human red blood cells were also assessed [5].

Two different cDNA sequences, have been described one encoding Lividin 8 with 95% identity to Lividin EV1 reported from *Odorrana exiliversabilis* (GenBank acc.

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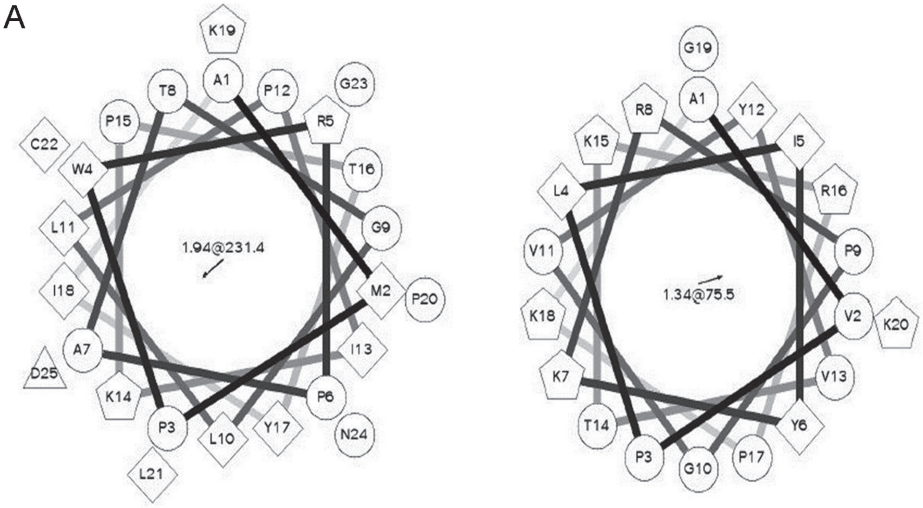
A



B

NCBI Accession NO	Publication Year	Current Name		Corrected Name
AIU99956	2014	Lividin-EV1	AVPLIYNRPGVYVTKRPKGK	Lividin 8 ODev
ADM34277	2010	Lividin-MT	AVPLIYNRPGVYVTKRPKGK	Lividin 8 AMma
AGG19129	2014	Odorranain-O-RA	AVPLIYNRPGIYVTKRPKGK	Lividin 8 AMmr
ADP06110	2010	Odorranain-O-RA	AVPLIYNRPGIYVTKRPKGK	Lividin 8 ODan
ACA81698	2007	Lividin-8	AVPLIYNRPGIYVTKRPKGK	Lividin 8 ODli
AEZ52986	2012	Lividin- OT	AVPLIYNRPSIYVTKRPKGK	Lividin 8 ODti
ACB05703	2009	Odorranain-O3	AVPLIYNRPGIYAPKRPKGK	Lividin 8 ODgr
Present Study		Lividin 8 HYba	AVPLIYKRPGVYVTKRPKGK	Lividin 8 HYba

A - 100% similar A - 80-99% similar A - 60-79% similar - A less than 60% similar

Fig. 1. Helical wheel and multiple sequence alignment of the peptides. A – Helical wheel diagrams of the peptides. The hydrophilic residues are represented as circles, hydrophobic residues as diamonds, negatively charged triangles, and positively charged as pentagons. B – Multiple sequence alignment of Lividin 8 peptides

no: ADV36193) and a second encoding Spinulosain precursor with 92% identity to Spinulosain-A1 from *Hylarana spinulosa* [7]. The new peptides were named according to the recently accepted nomenclature system [4] and submitted to GenBank (GenBank acc. nos: KR995268, KR995269). Both the peptides are cationic with +6 and +2 charges (which is the primary factor responsible for the interaction between biological membrane and peptide [6]), respectively and are hydrophilic (Table 1A). They exhibit weak antimicrobial activity against both Gram-positive and Gram-negative bacteria (Table 1B) and showed no haemolytic activity against human red blood cells even at a higher concentration of 175 μM. This could be correlated with negative GRAVY index (Table 1A) and helical wheel predictions (Fig. 1A) as there are no perfect demarcation of hydrophobic amino acids to one side of the helix and hydrophilic to the other side, which is against the characteristic feature of antimicrobial peptides. These results go parallel with previous report of Spinulosain from *H. spinulosa* [7], which did not show any antimicrobial activity. There were no previous reports on the activity of Lividin yet. Low antimicrobial activity of some skin derived peptides are hypothesized to be due to the presence of symbiotic bacteria on the skin surface of amphibians that play defensive roles, which can survive only in less toxic environment [2] or they may have antioxidant or synergistic action with other peptides [3]. However, the biological roles of such peptides, enhanced by post-translational modification or synthetic analogues could not be ruled out in a therapeutic context [6].

Table 1

Details of peptides identified from the skin secretion of *H. bahuvistara*

1A. Physicochemical properties of the peptides

	Number of amino acids	Net charge	GRAVY	Theoretical PI	Theoretical mass	Observes mass
Lividin 8HYba	20	+6	–0.540	10.66	2270.7	2270.7
Spinulosain HYba	25	+2	–0.132	9.20	2753.3	2753.3

1B. Minimal inhibitory concentrations of (MIC) of peptides against microorganisms

MIC(μM)	Lividin 8HLmb	Spinulosain HLmb
Gram-positive bacteria		
Staphylococcus aureus MTCC 9542	<200	<200
Bacillus subtilis MTCC 14416	NA	NA
Bacillus coagulans ATCC 7050	<200	NA
MRSA ATCC 43300	NA	NA
VRE ATCC 29212	NA	NA
Streptococcus mutans MTCC 497	NA	NA
Streptococcus gordonnii MTCC 2695	NA	NA
Gram-negative bacteria		
Vibrio cholerae MCV09	NA	NA
E. coli ATCC 25922	<200	<200

NA: Not appliable.

The name Lividin was first proposed for peptides isolated from *Odorrana livida* [8]. All the peptides isolated from *O. livida* were given the name Livdin without searching whether they had similarity with other reported peptides. Later, Lividin 1–4 were renamed as Brevinin and Esculentin [4] and proposed that the family name of the peptide should be selected giving priority to publication date. BLAST search gave seven peptides having 80–95% identity to the Lividin 8 HYba identified in the present study. However they are given different names irrespective of their structural similarity (Fig. 1B) as in the case of Gaegurin and Rugosin, which was renamed later [4]. Lividin 8 HYba identified in the present study showed similarity to Lividins and Odorranains, but on detailed analysis of their sequences and publication dates, it was found that such a sequence first reported was Lividin 8 (GenBank acc. no: ACA81698). Hence we recommend that all the peptides listed in Fig. 1B should be renamed as Lividin 8. The second peptide was named as Spinulosain without any confusion because on NCBI BLAST, it was found that only one peptide had >80% identical sequence (GenBank acc. no: ADV36193). We suggest, that before naming peptides, similar sequences should be searched in existing literatures and databases like NCBI and EMBL and their sequences be carefully analyzed.

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