Peptide similar to PR39 of frog skin *Hypsiboas pugnax* that evades the bacterial resistance of SAP-A in *E.coli* PR39 resistant

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Abstract

Colombia is the second country with the highest biodiversity in the world, and until now, 216 species of anurans have been reported in the department of Antioquia, including the tree frog *Hypsiboas pugnax*, whose importance in this study lies in their skin, because throughout the evolution of this tissue has become the first line of defense against microorganisms and predators given its ability to secrete an exudate with a wide bioactive molecules including peptides. In this study was assembled a transcriptome from mRNA of the skin of this tree frog using an Illumina sequencing platform Hiseq4000 obtaining 2,830,052 transcripts that were used to find peptides. Among these findings there is a similar PR39 peptide (Proline-Arginine-39) that is a small cationic, proline and arginine rich cathelicidin, which has been reported having multifunctional activities like wound healing, angiogenesis, antimicrobial and immunomodulation. However, pathogenic bacteria have evolved multiple strategies to resist and evade antimicrobial peptides, which is vital to bacterial survival and colonization in hosts. One of the important strategies for bacterial evasion of antimicrobial peptides involves the aid of transporter systems. The Sap transporter system is important for resistance to antimicrobial peptides in several Gram-negative pathogens.The objective of this work was to find whether similar PR39 peptide evades the Sap protein in *E.coli* resistant to PR39 through bioinformatics analysis.

Palabras Claves: Transcriptomic, PR39, peptide

Introduction

Colombia is the second country with the highest biodiversity in the world, currently 56,343 species have been reported, placing it in the first place in birds and orchids and in the second in plants and amphibians ("Biodiversity In Cifras - Sib Colombia"), In amphibians there are 803 species of which 367 are endemic and the highest percentage (93%) represent the order of anurans also known as batracios (Acosta et al., 2017). To date, 216 species of anurans have been reported in the department of Antioquia, including the tree frog Hypsiboas pugnax (Duellman et al., 2014). This frog belonging to the family Hylidae is also known as banana frog because you can observe it in regions where agroindustrial processes are carried out ("Illustrated guides of the River Porce Canyon").

The importance of the frog Hypsiboas pugnax in this study lies in the skin, because throughout the evolution this tissue has become the first line of defense against

microorganisms and predators for its ability to secrete an exudate with varied molecules bioactives that protect it from attack (König et al., 2015). Among these molecules are proteins, alkaloids, biogenic amines and peptides (Daly et al., 2000). The latter are important in the pharmaceutical market as they not only present a wide range of activities (antimicrobial, antitumor, wound healing) but also generate little bacterial resistance compared to conventional antibiotics (Tellez et al., 2010).

According to Xu et al., 2015 the majority (66%) of the peptides found in amphibians have antimicrobial activity, and are produced by the interaction of a pathogen and a membrane receptor in the epithelial cells of the frog skin, this triggers a cascade of secondary messengers that activate transcription factors that in turn generate the messenger RNA encoding an antimicrobial peptide (PAM) (Pushpanathan et al., 2013).

The peptide is produced as a prepropeptide which is not active until the signal region (pre) is removed by a signal peptidase and the acidic (pro) region by a serinprotease (Cooper, et al., 2010; Pushpanathan et al. al., 2013). The active peptide is released through the granular glands located in the dorsal region of the frog's skin to finally reach the microorganism and exert its cytolytic effect (Daly et al., 2000; Pushpanathan et al., 2013)

The cytolytic effect of the PAMs has two mechanisms of action, the first is known as membranolytic, is given through electrostatic interactions of the peptide with the membrane phospholipids of the microorganism triggering the formation of membrane pores (Hale et al., 2007) . The second mechanism is caused by invagination of the membrane of microorganisms that allows the entry of the PAMs into the cytoplasm and activate the release of cytochrome C from the mitochondria to initiate apoptosis and / or bind to DNA or RNA and block the processes of transcription and translation (Hale et al., 2007; Ageitos et al., 2017).

Wang et al., 2016 mentions that MAPs can be classified according to structure (α-helix, β sheet), biological source (amphibians, birds), biological function (antimicrobial, antitumor), synthesis (ribosomal, non-ribosomal) and physico-chemical properties (load, size, hydrophobicity). Within this latter classification are cationic peptides that have the best antimicrobial action with broad spectrum (gram +, gram-, fungi, viruses, parasites) due to their amphipathicity (50-60%), isoelectric point (10.8-11.8), (12-50 aa) and their propensity to form aggregates (Zeletzesky, 2006; Polanco, 2009; Ntwasa, 2012; Pushpanathan et al. 2013; Vishneposlky, 2014).

At present, there are two strategies for finding peptides in frogskin, peptidomics and transcriptomics, with the latter having advantages such as low cost, non-limiting sample quantity, identification of new genes, identification of microbiota of the skin and differential expression of genes and isoforms (Anamika et al., 2015, Chandramouli et al., 2009). Transcriptomes are generated from the transcripts of a tissue that has been sequenced through platforms such as the Hiseq4000 illumina / solexa that has advantages over other platforms because of its low error rate less

than 2% (Nagarajan, 2013). The transcriptome of this species will give a better understanding of its genetic background and will serve as a tool for future studies in this frog as in other hypsiboas genre.

Due to the biotechnological interest in the anurans, an importance is generated in the conservation of these animals and in the adequate utilization of their genetic resources, allowing also to improve the understanding of different aspects of their physiology (Ntwasa, 2012)

Objective

Finding whether similar PR39 peptide evades the Sap protein in *E.coli* resistant to PR39 through bioinformatics analysis

Methodology

Gathering and extraction

The skin of the frog was stimulated with the TAS (transcutaneous electrical stimulator) after wetting the animal with 1mL of deionized water, with the following parameters: 4-10 volts, 15-50 Hz frequency, 2-4 ms pulse for 10 -20 seconds. A cut was made in the dorsal and ventral region of each frog with a sterile scalpel to remove the tissue deposited in a 15mL Falco tube with Shield RNA from zymo research (tissue samples should not exceed 10% v / v) to conserve RNA and avoid contamination. Samples were stored at -70 ° C and sent to macrogen sequencing, South Korea.

Sequencing

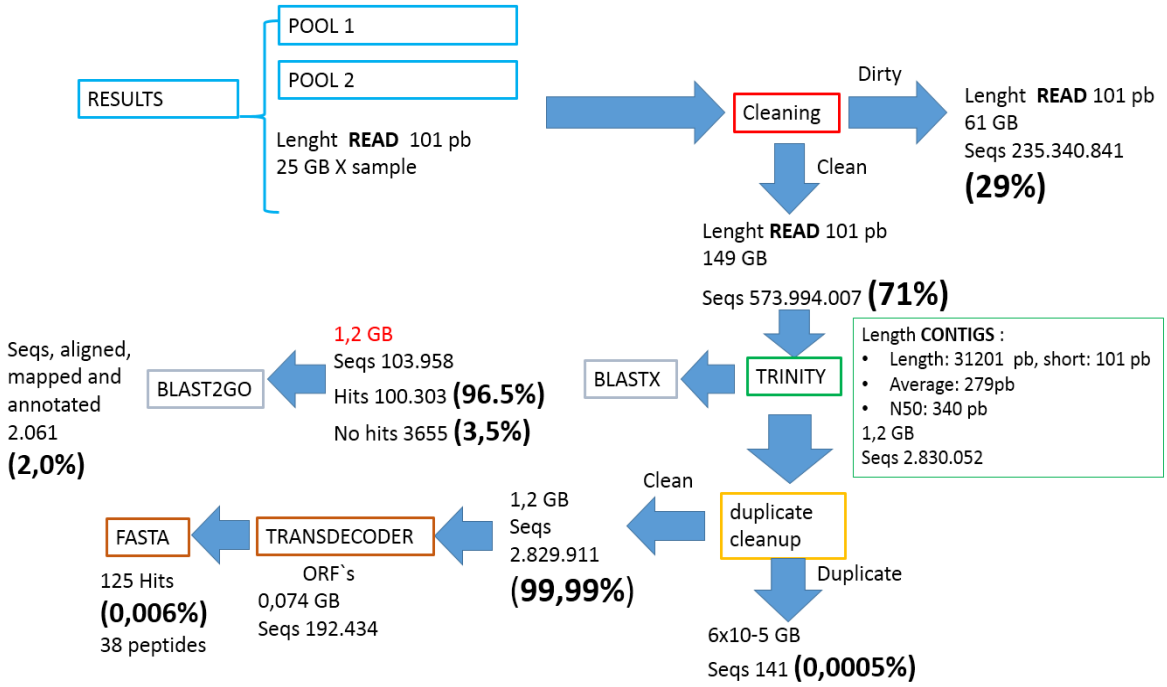
We performed the sequencing by synthesis of Illumina in the Hiseq4000 platform capacity 50GB memory (Gigabytes) with a depth 40x, the run type was paired end with reads length 2 x 100 base pairs. Previously the extraction of the messenger RNA with the ribozero kit was carried out.

Bioinformatic analysis

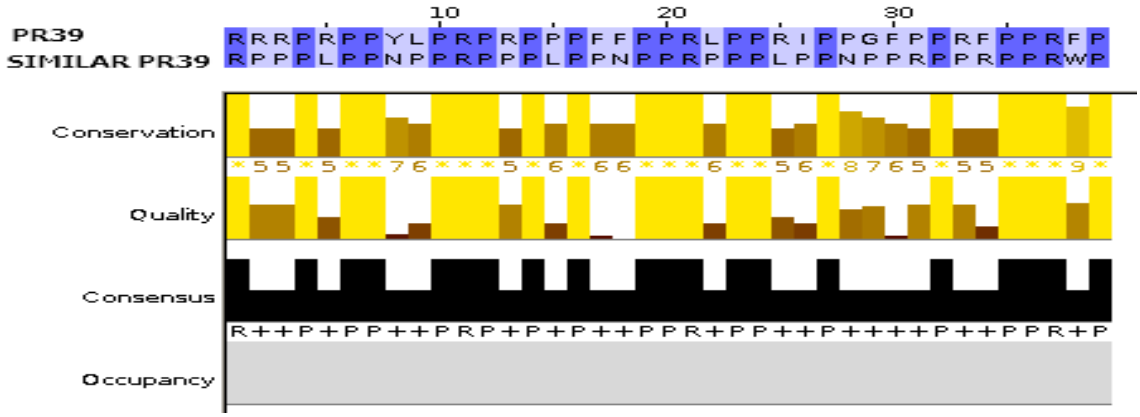
As it was sequenced in the paired end, that is to say the two ends for each reading originated 8 files in format fastq (quality format). These data were cleansed by taking read to other types of RNA (ribosomal RNA, RNA transfer and messenger RNA from other species) using SORTMERNA and also removed adapters using Cutadapt. The quality of the reading is checked with the FASTQC software. Subsequently the transcriptome was assembled again with TRINITY.

The superposition, surface features, hydrophilicity and distance calculations for individual structures were obtained using the PyMOL Molecular Graphics System (Schrödinger, OR) and the Chimera (University of California, San Francisco, CA) program.

Results



Comparison between PR39 and Similar PR39 Aligment



Comparison between PR39 and Similar PR39 physiochemical properties

Peptide	% Similarity	Cell location (psort)	Number of residues (pepcalcinnovagen)	Molecular weight (g/mol) (pepcalcinnovagen)	Iso-electric point (pH) (pepcalcinnovagen) (10.8-11.8)	Net charge at pH 7 (pepcalcinnovagen) (+4 a +9)	Water solubility (pepcalcinnovagen)	Grand average of hydropathicity (GRAVY) (Protoparam)	Instability index (Protoparam)	Hydrophobicity (Thermo scientific) (40-60%)	Cell penetrating peptide probability(C2Pred)	Structure Prediction (Psipred)
Similar PR-39	60	73.9 %: mitochondrial	39	4348.11	12.8	6	good	-1759	121.19 protein as unstable	37.29	0.86	no alpha helix no beta sheet
PR-39		56.5 %: mitochondrial	39	4720.63	12.7	10	good	-1308	118.68 as unstable	47.26	0.75	no alpha helix no beta sheet

Comparison between PR39 and Similar PR39 3D secondary structure

Conclusion

There similarities in structure and physicochemical properties between similar PR39 and PR39

This peptide found could have an antimicrobial and wound healing activity

PR39 similar could evade resistant mechanism SAP-A from PR39 resistant strain

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