Targeted amplicon sequencing for obtaining novel antimicrobial peptide sequences

Tomislav Rončević
Faculty of Science
University of Split

AARC 2017, Trieste, Italy, 28-30 August 2017
What are antimicrobial peptides...

Gene encode molecules with
- Direct *antimicrobial* activity
- High molecular *diversity*
- Mostly *cationic*

- Helical AMPs are among the most abundant and studied
...and where can we find them?

HDP = host defense peptides
- Endogenous peptides
- Co-evolved with microbes
- Innate and adaptive defenses

„...the term CHDPs has now been adopted to encompass the antimicrobial, immunological and other biological functions of these molecules...”

Taken from: http://aps.unmc.edu/AP/facts.php

AARC 2017, Trieste, Italy, 28-30 August 2017
Pharmaceuticals 2016, 9(3): 40; doi:10.3390/ph9030040
Development of antibiotics...
...and bacterial resistance

- Bacterial resistance was first observed in the 1940s
- MRSA was isolated 2 years after introducing methicillin

Taken from: http://pubchem.ncbi.nlm.nih.gov/compound/5311054#section=Top

Taken from CDC report: ‘Antimicrobial Resistance Threats in the United States’, 2013

AARC 2017, Trieste, Italy, 28-30 August 2017
How to fight „superbugs” (multiresistant bacteria)?
Possible alternative – frog AMPs

„Classical approach“:

• Treat the frogs with electric shocks or norepinephrine (noradrenaline)

• Extract and purify by precipitation and chromatographic techniques

• Activity testing of fractions to identify the active principles
Targeted amplicon sequencing

Quick look at the AMP structure:

- **SIGNAL PEPTIDE**: MFTLKKSLLLLFFLGTINLSLC
- **PROPEPTIDE**: MFTLKKSLLLLFFLGTISLSLC
- **MATURE PEPTIDE**: MFTMKKSLLLLFFLGTISLSLC

Highly conserved!

- **SIGNAL PEPTIDE**: FLPLAVSLAANFLPKLFCKITKKC
- **PROPEPTIDE**: IIPLPLGYFAKKT
- **MATURE PEPTIDE**: GVIDALKGAAKTVAELLRKAHCKLTNSC

Highly diverse!
Targeted amplicon sequencing

1) Obtain single individuals from the frog species of interest for which signal sequences are available in DADP database (e.g. Ranidae family)

Ranidae:

- *Pelophylax kl. esculentus* (Linnaeus, 1758)
- *Pelophylax ridibundus* (Pallas, 1771)
- *Rana arvalis* (Nilsson, 1842)
- *Rana dalmatina* (Fitzinger in: Bonaparte, 1838)
- *Rana temporaria* (Linnaeus, 1758)
Targeted amplicon sequencing

2) Assemble transcriptomes of those species available in SRA (Sequence Read Archive) database which are closely related to the target species (e.g. pertaining to the same family)
Targeted amplicon sequencing

3) Derive a consensus signal sequence based on the HMM profile

4) Translate transcripts to all six possible reading frames

5) Screen translated transcripts with consensus signal sequences and obtain 1st batch of potential AMPs

6) Based on the signal peptide regions of acquired AMPs design degenerate primers (reverse primer designed on the poly-A tail of mRNA)
Targeted amplicon sequencing

7) Isolate RNA from skin tissue

8) Synthesize cDNA library and perform PCR amplification

9) Size-select resulting amplicons and proceed to ion-semiconductor sequencing → assemble reads into contigs → translate to amino acid sequence → 2nd batch of potential AMPs

http://www.openwetware.org
https://www.nobelprize.org

AARC 2017, Trieste, Italy, 28-30 August 2017
Future steps...

10) Choose the most suitable peptides for synthesis and proceed to extensive characterization, structure, microbiological activity, mode of action, toxicity etc.
THANK YOU FOR YOUR ATTENTION !!!