rAMPage: Rapid Anti-microbial Peptide Annotation and Gene Estimation

Diana Lin[1,2], Ka Ming Nip[1,2], Sambina Aninta[1], Chenkai Li[1,2], René L. Warren[1], Caren Helbing[3], Linda Hoang[4,5], Inanc Birol[1,6]

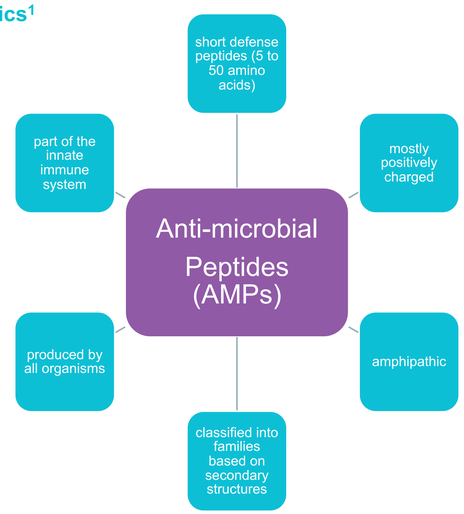
[1]Canada's Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, BC, Canada
[2]Bioinformatics Graduate Program, University of British Columbia, Vancouver, BC, Canada
[3]Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada
[4]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada
[5]Public Health Laboratory, British Columbia Centre for Disease Control, Vancouver, BC, Canada
[6]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada

Contact: dlin@bcgsc.ca
https://github.com/dy-lin

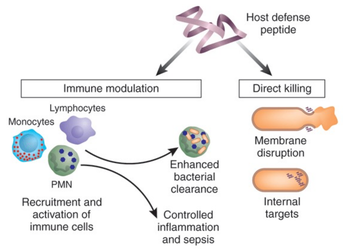
Introduction

Anti-microbial Peptides (AMPs)

Characteristics[1]

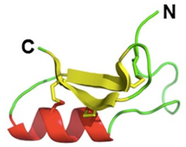


Mechanisms of Action[1]

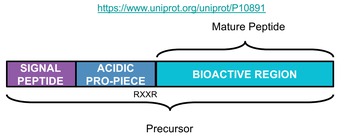


Source: Hancock, R. E. W. & Sahl, H.-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat. Biotechnol. 24, 1551–1557 (2006) doi: 10.1038/nbt1267

Peptide Structure[1]



Above: Insect Defensin A (phormicin) from the Northern blowfly
https://www.rcsb.org/structure/1ICA
https://www.uniprot.org/uniprot/P10891



- AMPs are activated by cleavage at the RXXR motif (acidic propiece inhibits basic bioactive region)
- Cleavage separates the signal peptide and acidic propiece from the bioactive region yielding the mature peptide

Motivation

PROBLEM antibiotic resistance → **SOLUTION** antimicrobial peptides

Problem

- The rise of antibiotic resistance[1]
- The antibiotic "discovery" void[2]: few new antibiotics, but old antibiotics less effective
- The need for novel methods to fight pathogen

Solution

- AMPs do not confer resistance as easily as antibiotics, due to co-evolution with the human microbiome[1]
- AMPs are a potential alternative to antibiotics[3]
- AMPs can be mined from organisms of rich AMP diversity, such as the North American bullfrog[4]

Objectives

- To develop and execute a scalable bioinformatics-based AMP discovery pipeline (i.e. *rAMPage*) to mine for AMP sequences in publicly available genomic resources
- To package a fully functional bioinformatics pipeline
- To obtain a list of potential AMP sequences for
  - Downstream analysis
  - *In vitro* bioactivity testing
  - Drug development

INPUT Publicly available RNA-seq reads[5] → *rAMPage* → **OUTPUT** Potential AMP protein sequences

Methods

Pre-process Reads
- Trim to remove adapter sequences using fastp[6]
- Filter out poor-quality bases and sequences
- fastp finds adapter sequences using sequence overlap

Assemble Transcripts
- Multi-sample pooled *de novo* or reference-guided assembly of reads into transcripts using RNA-Bloom[7], for both single and paired-end reads
- Lowly-expressed transcripts are filtered out using quantification from Salmon[8]

Translate Transcripts
- *In silico* translation of transcripts into amino acid space
- Six-frame translation and open reading frame (ORF) prediction using Transdecoder[9]

Homology Search
- Homology search with *jackhmmer* from the HMMER[10] package, using AMP databases APD3[11] and DADP[12]

Cleave Precursors
- Predict signal peptide and propeptide cleavage sites using prediction tool ProP[13]
- Separate signal peptide and acidic propieces from the bioactive region

Prioritize AMPs
- Obtain the probability that each sequence is an AMP using AMPlify[14]
- Rank potential AMPs and prioritize which sequences are synthesized and tested *in vitro* for bioactivity

Annotate AMPs
- Annotate AMPs using E_NTAP[15] to determine known gene ontologies and protein domains
- Determine AMP novelty by alignment to known AMPs using Exonerate[16]

Characterize AMPs
- Predict secondary structures and solvent accessibility using SABLE[17]
- Using SABLE, alpha helix and beta strand structures can be predicted

Results

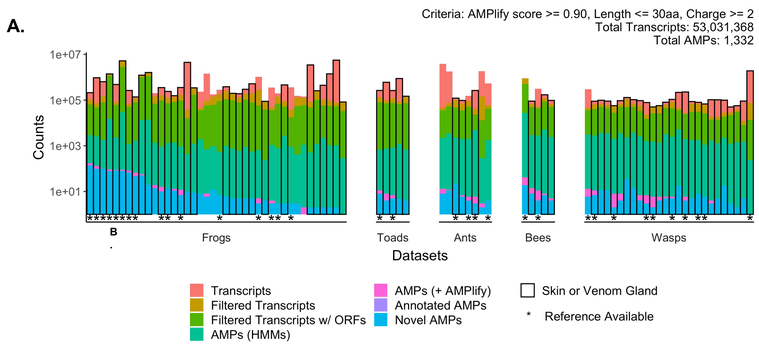


Fig A.[18] Count progression from transcripts to AMPs. Across the 84 datasets, *rAMPage* assembled > 53 million transcripts, and detected > 1000 putative AMPs (AMPlify score >= 0.50 is an AMP; stricter criteria used above).

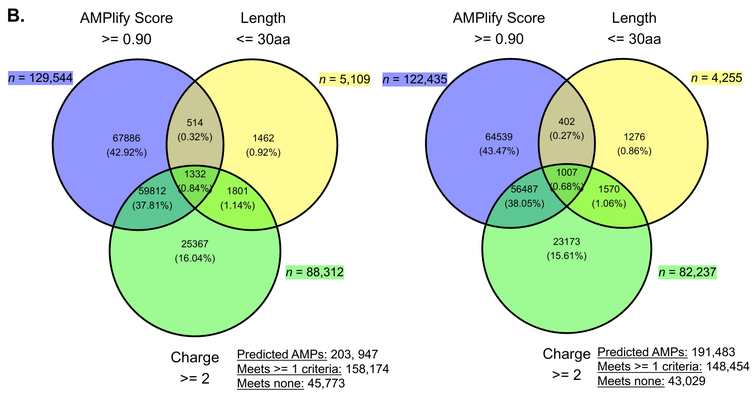


Fig B.[19] AMP counts after applying three filters. Three strict filters (AMPlify score >= 0.90, length <= 30aa, charge >= 2) are applied in *rAMPage*. [Left] 1,332 AMPs remain after filtering and duplicate sequence removal *within each* dataset. [Right] 1,007 AMPs remain after filtering and duplicate sequence removal *across all* datasets. If desired, more AMPs (of lower confidence) can be detected by adjusting the stringency of the filters.

C. *rAMPage* Benchmarking

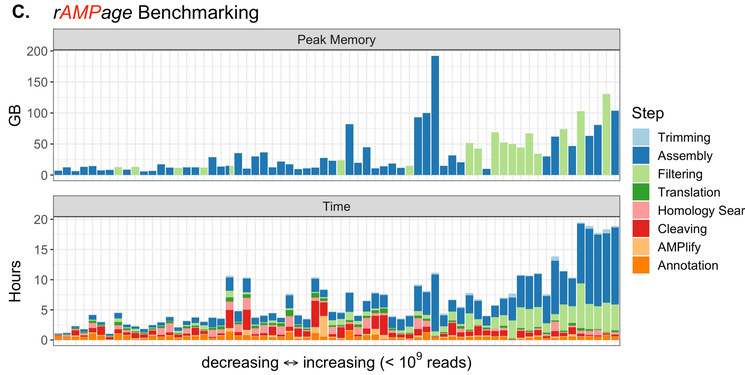


Fig C.[18] Runtime and memory usage of *rAMPage*. *rAMPage* is fast: with < 1 billion reads (74/84 datasets), results can be obtained within 24 hours, using < 200 GB of memory. Larger datasets with > 1 billion reads can be subsampled to reduce runtime and memory usage.

Conclusions

- Across the 84 assembled transcriptomes, 1,007 confident (AMPlify score >= 0.90), short (length <= 30aa), and positive (charge >= 2) unique mature putative AMPs were found: 795 from amphibians, 212 from insects
- Of these 1,007 AMPs, 254 sequences align to known AMPs with 100% sequence identity in the mature region; 753 sequences are 'novel' AMPs
- *rAMPage* is a fast, robust bioinformatics pipeline that, given raw reads, can detect known and novel putative AMPs

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