Analysis of the skin secretion of *Leptodactylus labyrinthicus*, the frog's biohazard protective clothing

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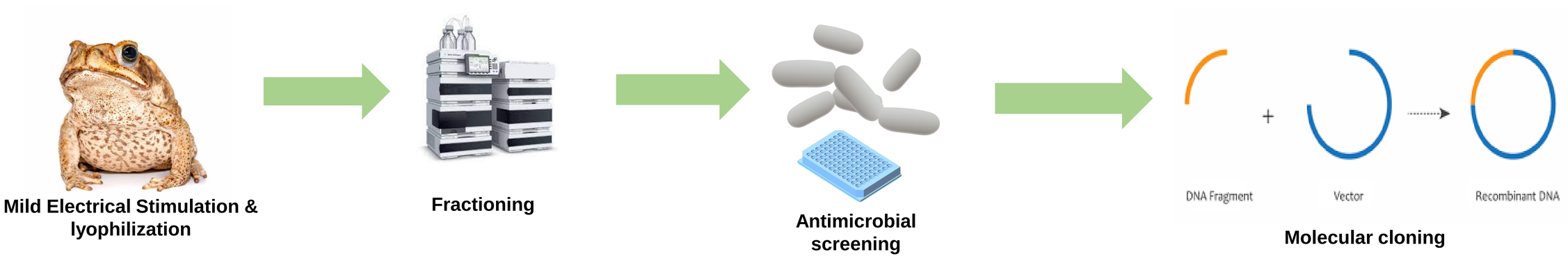
Introduction

The skin secretions of amphibians have long time been used in a variety of contexts, from religious ceremonies to agro-economical applications and medical treatments. These secretions consist of a mixture of different molecules, but especially the constituent of active peptides has been studied intensively during the past 30 years. Peptides have shown to be able to inhibit the growth of several human microorganisms (making from them good candidates for therapeutic agents), as well as soil microbes.

Leptodactylus labyrinthicus (Spix, 1824) (Anura: Leptodactylidae) is a large neotropical frog that inhabits central and southeastern Brazil, northeast Argentina and eastern Paraguay (Frost, 2017), and occurs from sea level up to 1000 meters of altitude (Heyer et al., 2004). This species seems relatively tolerant to pollution (Carvalho et al., 2013), and thus can be found in many anthropogenically impacted habitats.

The **objective** of this work was to analyze the secretion of *Leptodactylus labyrinthicus* in order to identify bioactive peptides, and to examine the biological activity of these molecules in an ecological context.

Methodology



Results

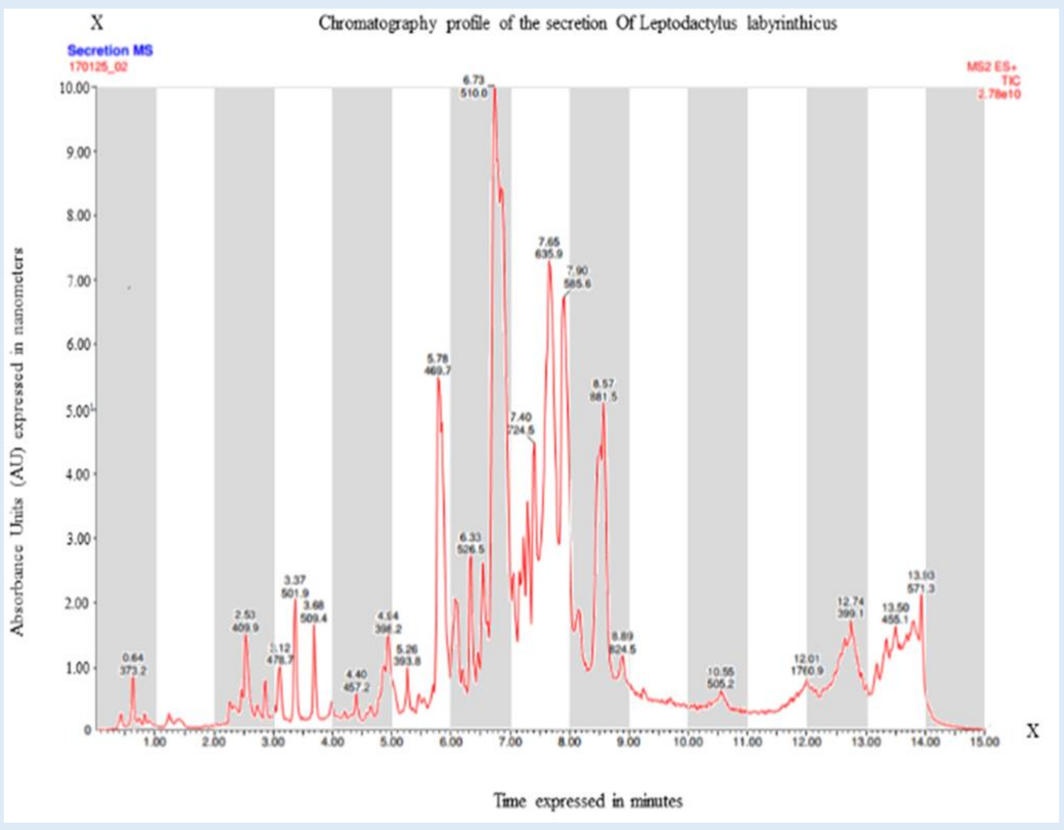


Figure 1. Chromatographic profile of the skin secretion of *Leptodactylus labyrinthicus*. Each column represents a fraction. The X axis represents time (minutes), the Y axis intensity (nanometers). The numbers above the peaks represent the molecules' retention time (the time when they eluted, upper number) and their molecular mass (lower number).

Bacteria	Fractions	Whole secretion	Negative control
Staphylococcus aureus	**8**	X	X
Burkholderia cepacia	**8-9**	✓	X
Escherichia coli	X	X	X

Table 1. Effect of *L. labyrinthicus* secretion (or fractions of it) on bacterial growth. Numbers indicate fractions slightly delaying the growth, the tick represents promotion of growth, the cross represents no effect at all (no delay nor promotion of growth).

Molecular cloning sequencing

MVKKPLLLVLLFGMVSLSICDEERRQDDEDDEEDDEEKR**GLLDTLKGAAKNVVGSLASKVMEK**

LG*; Mw (average mass): 2543.06; Mw (monoisotopic mass): 2541.46.

Sequence 2:

MLKKPLFLVLFLGLVSLSICDEEKREDEDDEEEDEDEEKR**GVVDILKGAAKDIAGHLASKVMNK**

LG*; Mw (average mass): 2549.07; Mw (monoisotopic mass): 2547.46.

Figure 2. Mature peptides are underlined with a single line and bolded, putative signal peptides are underlined with a double line, and stop codons are indicated by asterisks.

Discussion & Conclusion

Of the 15 fractions that were obtained by HPLC, only fraction n° 8 had a lightly inhibitory/delaying effect over the growth of *S. aureus;* fractions 8 and 9 slightly delayed the development of *B. cepacia*. The skin secretion tested in its entirety, showed a bacteriostatic effect on *S aureus*, but promoted the growth of *B. cepacia*. In nature, amphibian skin peptides act in concert, not separately, so this the positive effect on the growth of *B. cepacia* may be functionally significant.

We hypothesize a mutualistic relationship between bacteria and the amphibian, with *L. labyrinthicus* refraining from investing energy in the production of a bactericide secretion/peptides. This would also free energy to invest in other metabolic, ecological or physiological processes.

Further studies are necessary to understand which factors determine the potentiality of the peptides, if there exists a synergy between the molecules, and whether this synergy could bring some benefits for the animal.