

B1CTcu5 analogs as promising antimicrobial peptides, replacing the sequence cysteine

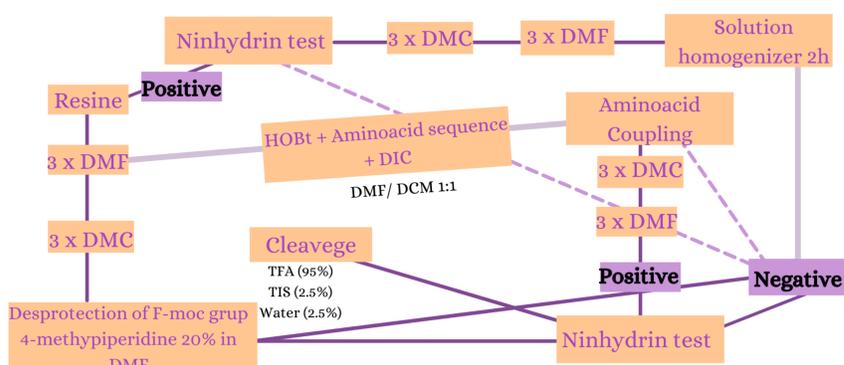
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Introduction

Due to the increase in emergency diseases and bacterial resistance and the drop in the development of new drugs, it has become necessary to develop solutions for the treatment of these pathologies. AMPs have several mechanisms to combat pathogens, as their actions are performed from direct contact with bacterial membranes through the formation of amphipathic structures with the hydrophilic and hydrophobic regions in the opposite parts of the peptides. The B1CTcu5 peptide was originally isolated from the cutaneous secretion of the Indian frog, *Clinotarsus curtipe*, belongs to the Brevinin-1 family and has been shown to have potential activity against infectious bacteria. In our research, homologues of B1CTcu5 were synthesized due to the fact that in its original sequence it contains cysteine that present spontaneous reactions due to the presence of thiol groups, making it difficult to understand the exact sequence that shows the activity; to test the antimicrobial activity of the homologues, the bacteria *Salmonella Typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were used.

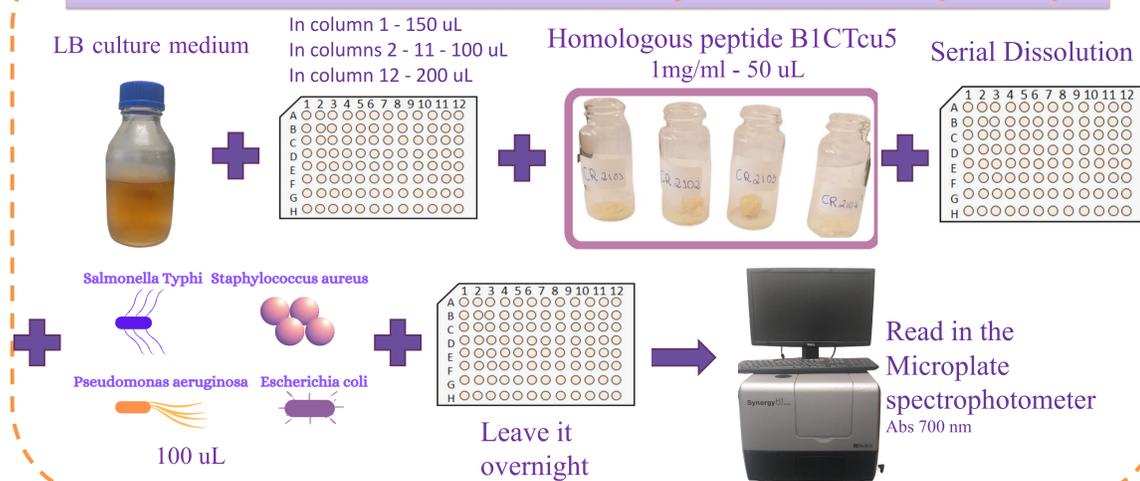
Material and Methods

Solid Phase Peptide Synthesis (SPSS)



- 1) LIAGLAANFLPQILSKIARKA
- 2) LIAGLAANFLPQILSKIARKS
- 3) LIAGLAANFLPQILSKIARKK
- 4) LIAGLAANFLPQILKKIARKS.

Determination of Minimum Inhibitory Concentration (MIC90)



Results and Discussion

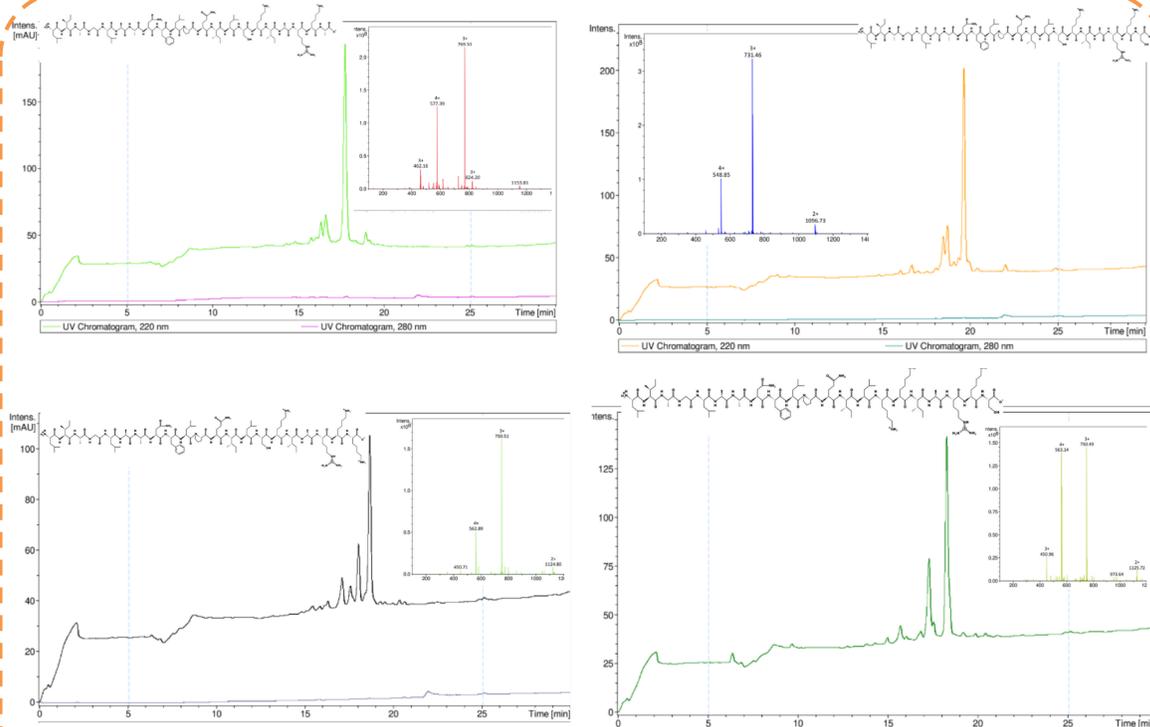


Figure 1: HPLC-MS analysis of the LC-MS analysis; MS spectra and aminoacid sequence of B1CTcu5 homologues (antimicrobial peptide)

Purity of the peptide was 95% assessed by HPLC and confirmed by LC-MS

References

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MIC-values

| Compound | <i>S. Tiphymurium</i> | <i>P. aeruginosa</i> | <i>S. aureus</i> | <i>E. coli</i> |
|----------|-----------------------|----------------------|------------------|----------------|
| 1-analog | 31,25 | > 62,5 | 125 | > 62,5 |
| 2-analog | > 31,25 | > 62,5 | 62,5 | > 62,5 |
| 3-analog | > 31,25 | > 62,5 | 62,5 | > 62,5 |
| 4-analog | > 15,625 | > 62,5 | 31,25 | > 62,5 |

Table 1: MIC values of B1CTcu5 homologues (antimicrobial peptide) against *S. Tiphy*, *P. aeruginosa*, *S. aureus*, *E.coli*

All chromatographic profiles are shown in their impure form (just after cleavage), and mass spectrometric analysis represents the peak and retention time of the purified peptide. The purity of the peptide was greater than 95% and used for microbiological studies. The minimum inhibition concentrations in 96-well micro-silver were performed in triplicate. MIC-values between 15.6 - 62.5 µg/mol were obtained, which shows the efficiency of substituting cysteine for other amino acids such as alanine and lysine.

Conclusion

These result show that the B1CTcu5 analogs could possess an effective antimicrobial activity using variations and replacing the sequence cysteine.

Acknowledgments

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