Determination of the 3D Structure of an Antimicrobial Peptide: Subtilosin A

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HISTORY

- Subtilosin A is an peptide isolated from bacteria that has antimicrobial activity.
- This antimicrobial peptide could be used in both food production and pharmaceutical industries.
- Antimicrobial peptides are used for biopreservation in the food which helps lower the costs of processing as well as increasing food safety.
- As well as being applied in the food industry, some peptides can also be used in medicine due to their antimicrobial properties.
- Due to the overuse and incorrect use of antimicrobial drugs, an increase in antimicrobial resistance has been observed. Pharmaceutical companies have not been able to respond well to this fast increase in resistance.
- Subtilosin A is being studied as a potential alternative to the limited antibiotic resources we currently have.

OBJECTIVE

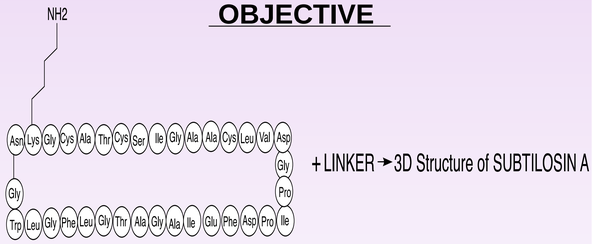
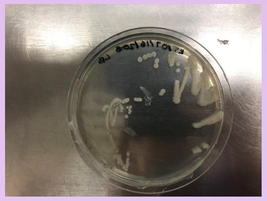


Figure 1: Schematic Representation of the Objective

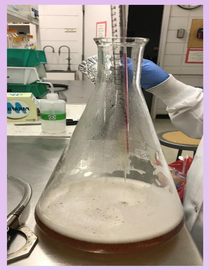
- The objective of this project is to determine the structure of the peptide, subtilosin A, by X-ray crystallography.
- The first step is the expression and purification of subtilosin A. Our current method involves cell culturing by plate streaking and inoculation, butanol extraction and HPLC purification.
- The next step will involve the chemical attachment of a linker to the purified peptide, as shown is Figure 1 above.

METHODS

1) Bacterial Growth and the making of 1 L Culture Flasks

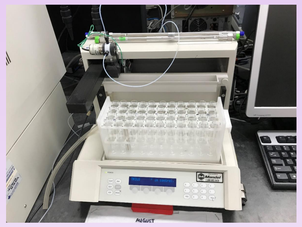


2) Inoculation



3) Butanol Extraction and Evaporation

4) HPLC Purification



RESULTS

- This figure shows a peak around the 18.3 minute mark on the HPLC machine. Fractions 7 and 8 were collected and tested using the MALDI (Matrix Assisted Laser Desorption/Ionization) to determine the molecular weight.

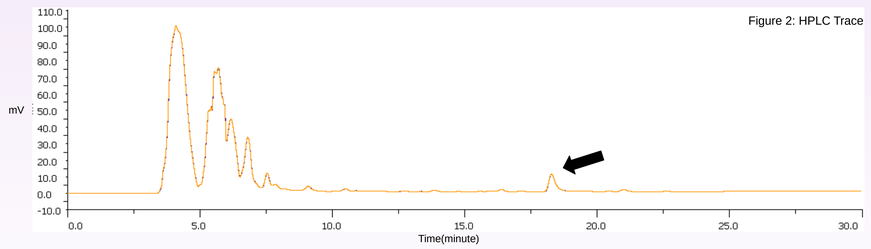


Figure 2: HPLC Trace

- This spectrum indicates there is a peak at 3402 m/z, which is the molecular weight of subtilosin A.

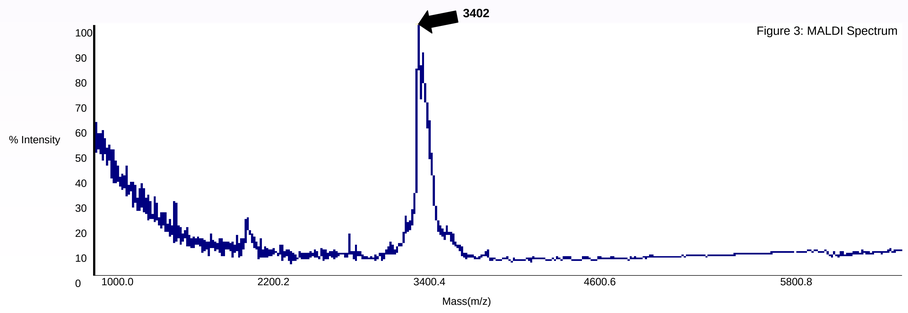


Figure 3: MALDI Spectrum

CONCLUSIONS

- The HPLC purification is required to purify the peptide and MALDI analysis is required to identify the molecular weight of our desired peptide.
- The expected molecular weight of subtilosin A, 3402 g/mol, was observed in the MALDI spectrum. The lack of any other peaks confirms that this is a pure sample.
- Both of these steps, HPLC and MALDI, are necessary in order for us to ensure that the peptide is in our sample and we are able to go forward with future directions.

FUTURE DIRECTIONS

- Now that we have isolated a pure sample of subtilosin A, we can go forward with the next step of our objective.
- In order to further the objective, future advances involve linker chemistry which will attach subtilosin A to another protein. We hypothesize that this will allow us to crystalize subtilosin A and determine its 3D structure.

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CITED LITERATURE

- Balciunas, E. M., Martinez, F. A., Todorov, S. D., Franco, B. D., Converti, A., & Olivieira, R. P. (2012, November 27). Novel biotechnological applications of bacteriocins: A review. *Food Control.* **2013**, *32*, 134-142.
- Kawalka, K., Sprules, T., Diaper, C. M., Whittal, R. M., McKay, R. T., Mercier, P., Zuber, P., & Vederas, J. C. Structure of subtilosin A, a cyclic antimicrobial peptide from *Bacillus subtilis* with unusual sulftur to alpha-carbon cross-links: formation and reduction of alpha-thio-alpha-amino acid derivatives. *Biochemistry.* **2004**, *30*, 3385-3395.