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Learning the Science Behind Bacteriocins Through Lacticin 3147; a Promising Lantibiotic

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Abstract

As microorganisms continue to develop resistance and survive against many different forms of antimicrobial solutions, such as antibiotics, the threat that antimicrobial resistance poses grows considerably. One solution to this persistent issue could be bacteriocins: ribosomally synthesized antimicrobial peptides that are synthesized by bacteria. In this study, the specific lantibiotic—a subclass of bacteriocin—used was lacticin 3147, which is comprised of two components: A1 and A2. Lacticin 3147 was first purified and isolated in order to properly analyze its antimicrobial effects, which show potential use in antibiotics or food preservation. The procedure started with growing the producer bacteria strain, Lactococcus lactis DPC 3147 in a broth which was later used to inoculate a large volume of media. This media was then separated through centrifugation into two components: the supernatant and cell pellet, both of which were each individually concentrated and purified through a series of columns. Approximately one milliliter of each component was run through a High Performance Liquid Chromatography (HPLC) machine, and the resulting chromatograms interpreted to evaluate and compare the concentrations of lacticin 3147 produced in the liquid media portion (supernatant) and the cell components (cell pellet). Subsequently, fractions were collected from all runs from the HPLC and further subjected to Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI TOF) mass spectrometry. This allows to test the molecular weights of the compounds in the samples to check if they aligned with the known molecular weights of both the A1 and A2 components of lacticin 3147. The final step was to prepare a spot on lawn assay using the indicator species: Lactococcus lactis subsp. cremoris HP. The spot on lawn assay prepared for lacticin 3147 was a visual indicator of the strong antimicrobial effects of the bacteriocin. Ultimately, this highly effective bacteriocin, lacticin 3147, could be utilized in smaller concentrations than current antibiotics, and thus shows great promise in the field of antibiotics. Further studies are being conducted to understand the interactions between the A1 and A2 components of lacticin 3147, including their synergistic effects.

Key words:

Bacteriocin, Lacticin, bacteria, Lactococcus lactis, Lactococcus lactis bacteria, antimicrobial resistance, purification, purification of bacteria

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Abstract

- In 2017, antimicrobial resistance was declared a global concern by the WHO.[1]
- It is predicted that in 2050, ten million people around the world will die as a result.[1]
- Antimicrobial resistance is the developed ability of microorganisms to survive against antibacterial solutions.[1]
- Bacteriocins are antimicrobial peptides that are ribosomally synthesized by bacteria which show great promise for use in food preservation as a replacement for antibiotics.[2]
- Lacticin 3147 is a two component (Ltn A1, Ltn A2) lantibiotic, a subclass of bacteriocins, produced by the bacteria *Lactococcus lactis* subsp. DPC 3147.[3]
- Because of its broad activity on a range of Gram-positive bacteria and stability compared to nisin, a broadly used bacteriocin, lacticin 3147 became the focus of many studies.[4]

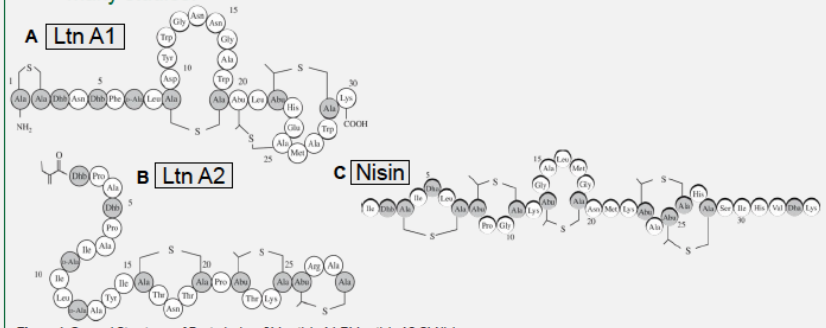

Figure 1, General Structures of Bacteriocins. **A)** Lacticin A1 **B)** Lacticin A2 **C)** Nisin

Lacticin 3147 Mechanism of Action

- Studies suggest Ltn A1 binds to the outer membrane component lipid II and recruits Ltn A2, subsequently lysing the cell.[5]

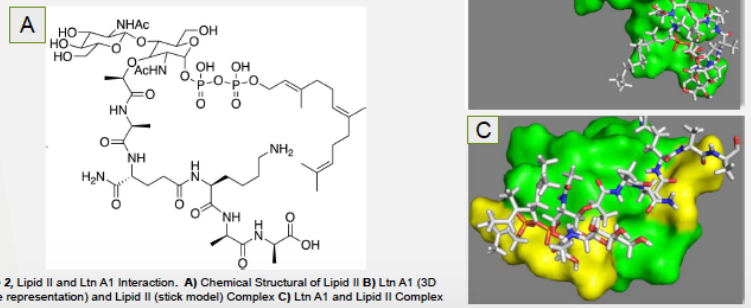

Figure 2, Lipid II and Ltn A1 Interaction. **A)** Chemical Structural of Lipid II **B)** Ltn A1 (3D surface representation) and Lipid II (stick model) Complex **C)** Ltn A1 and Lipid II Complex

Purification General Methods

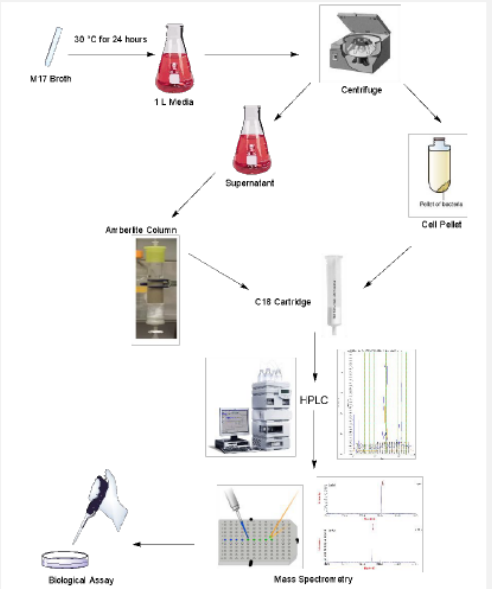

Figure 3, General Lacticin 3147 Guided Purification Process

Conclusion

- Both the A1 and A2 structures found in Lacticin 3147 are active in small concentrations (~μM/mL – nM/mL), and therefore have strong antimicrobial effects
- Further studies being conducted involving isotope labelling of A1 and A2 for deeper mechanism of action studies
- Bacteriocins are a promising field of study to fight against antimicrobial resistance

Results

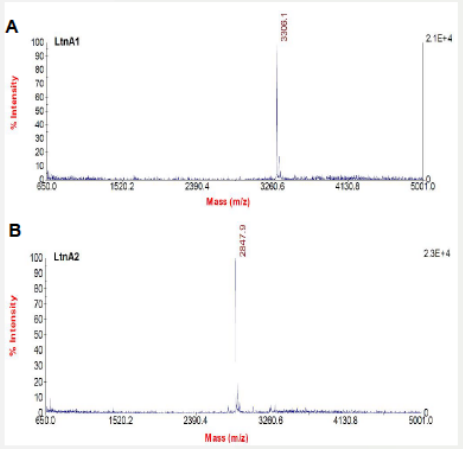

Figure 4, MALDI-TOF spectra. **A)** Ltn A1 **B)** Ltn A2

HPLC chromatogram results suggest that productivity in the cell pellet was greater than the supernatant.

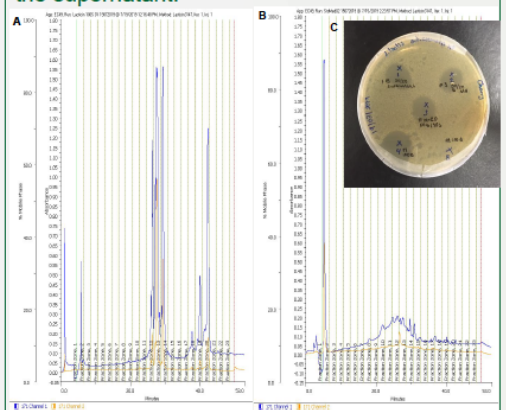

Figure 5, HPLC Chromatogram. **A)** Cell Pellet **B)** Supernatant **C)** Spot on Lawn Assay Against *L. lactis* subsp. cremoris HP

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