4-21-2023

α-Hemolysin as a Candidate for a Vaccine for *Staphylococcus aureus* in Bovine Mastitis

Madeline B. Clark
Boise State University

Juliette K. Tinker
Boise State University

α-Hemolysin as a Candidate for a Vaccine for *Staphylococcus aureus* in Bovine Mastitis

Abstract

Staphylococcus aureus is a Gram-positive bacteria responsible for many types of infections. It is abundant in nature, even present on our own skin, usually harmless. However, it is the leading cause of infection in humans. S. aureus also harms animals, and in dairy cows, causes Bovine mastitis. This disease results in a decreased quality and quantity of milk, inflammation of the mammary glands, and can even be transmitted to humans.(1) Because of this, there are massive economic ramifications estimated at $629 million annually.(2) This study focuses on a virulent factor known as a-hemolysin (Hla) and cloning this into S. aureus bacteria to make a vaccine to treat bovine mastitis. This is a protein present on the cell membrane of S. aureus, known for its cytotoxic properties. To harm eukaryotic cells, research suggests that Hla has a close relationship with a eukaryotic cell receptor known as ADAM10. Normally, this receptor has a role in the development of the nervous system, and in precursor formation of the amyloid protein. When S. aureus is exposed to these cell receptors, a bridge is formed between the Hla protein of the bacteria and the surface receptor ADAM10. After the link is formed, the Hla protein drills a pore into the eukaryotic cell causing it to lyse. (3) This makes the Hla protein a great candidate for a vaccine, as if this interaction could be prevented, then harm would be reduced in the host cell.

α-hemolysin as a Candidate for Vaccine for *Staphylococcus Aureus* in Bovine Mastitis

Madeline B. Clark, Juliette K. Tinker
Department of Biological Sciences, Boise State University

BOISE STATE UNIVERSITY

Introduction

Staphylococcus aureus is a Gram-positive bacteria responsible for many types of infections. It is abundant in nature, even present on our own skin, usually harmless. However, it is the leading cause of infection in humans. S. aureus also harms animals, and in dairy cows, causes Bovine mastitis. This disease results in a decreased quality and quantity of milk, inflammation of the mammary glands, and can even be transmitted to humans.(1) Because of this, there are massive economic ramifications estimated at $629 million annually.(2) This study focuses on a virulent factor known as **α-hemolysin** (Hla) and cloning this into S. aureus bacteria to make a vaccine to treat bovine mastitis. This is a protein present on the cell membrane of S. aureus, known for its cytotoxic properties. To harm eukaryotic cells, research suggests that Hla has a close relationship with a eukaryotic cell receptor known as ADAM10. Normally, this receptor has a role in the development of the nervous system, and in precursor formation of the amyloid protein. When S. aureus is exposed to these cell receptors, a bridge is formed between the Hla protein of the bacteria and the surface receptor ADAM10. After the link is formed, the Hla protein drills a pore into the eukaryotic cell causing it to lyse. (3) This makes the Hla protein a great candidate for a vaccine, as if this interaction could be prevented, then harm would be reduced in the host cell.

Methods and Results

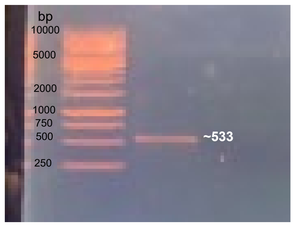

Figure 2: PCR/ Gel Electrophoresis of Hla

Successful sample 1 PCR next to a 10000 bp ladder. Hla protein is ~533 bp long. Sample 2 was unsuccessful.

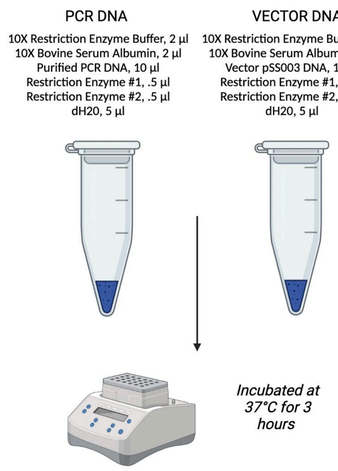

Figure 3: Restriction Digest

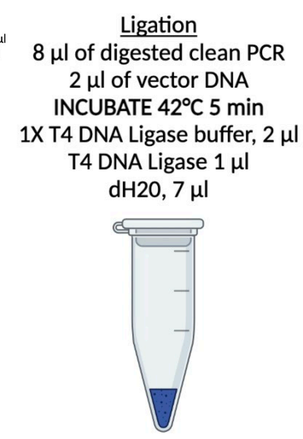

Figure 4: Ligation

Bioinformatics

Uniprot Information: Locator SAOUHSC_01121

- Protein Function: *Alpha-toxin binds to the membrane of eukaryotic cells (particularly red blood cells, RBC) forming pores, resulting in hemolysis, with the release of low-molecular weight molecules leading to eventual osmotic RBC lysis." (4)*
- Protein Location: SAOUHSC_01121 , and found on surface membrane

Amino Acid Sequence

MKTRIVSSVTTTLLLGSILMNPVAGAADSDINIKTGTTDIGSN
TTVKTGDLVTYDKENGMHKKVFYSFIDDKNHNKKLLVIRTKG
TIAGQYRVYSEEGANKSGLAWPSAFKVQLQLPDNEVAQISD
YYPRNSIDTKEYMSTLTYGFNGNVTGDDTGKIGGLIGANVSI
GHTLKYVQPDFKTILESPTDKKVGWKVIFNNMVNQNWGPY
DRDSWNPVYGNQLFMKTRNGSMKAADNFLDPNKASSLLSS
GFSPDFATVITMDRKASKQQTNIDVIYERVRDDYQLHWTST
NWKGTNTKDKWTDRSSERYKIDWEKEEMTN

Figure 1: *3D Structure of Hla*

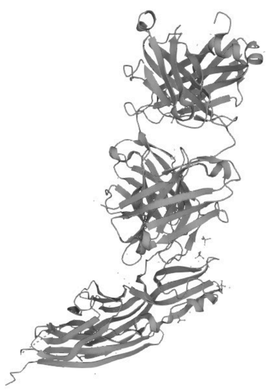

Table 1. Whole Genome Shotgun Homology/ BLAST

Biocode	Strain	% Coverage	% Identity
EFW32871.1	Mrsa 131	99%	100%
EMS38056.1	KLT 6	99%	100%
EFK81723.1	TCH70	99%	99.69%
AGU61224.1	CN1	99%	99.37%
EhS70170.1	IS-125	99%	99.37%

Vaxign Results

- ML Score: 98.5
- # MHC I: 16
- # MCH II: 4
- Adhesion probability: .636
- Homology to humans: none
- # transmembrane helices: 1

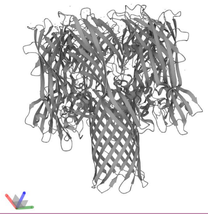

Figure 5: *3D Structure of Hla pore complex*

- Protein Structure: "*The mushroom-shaped heptamer is composed of a cap domain,7 rim regions, and the stem domain which forms the transmembrane pore." (4)*

Conclusion

α-hemolysin is a great candidate for a vaccine as it plays a critical role in the pathway S. aureus has for harming eukaryotic cells. Hla sample 1 had a successful PCR and went on to be digested and then ligated. To proceed forward, a transformation will be completed to have the protein incorporated into S. Aureus DNA. Those cells will be lysed, and then PCR performed to see if they took up the new DNA.

Bioinformatics conclusion: The Hla protein was shown to be virtually identical in other strains of S. Aureus, confirming that this would be an appropriate target for a vaccine. The protein also showed significant Vaxign results about the nature of Hla. Most importantly, the cytotoxic properties Hla possesses lets it lyse eukaryotic cells. By preventing this pathway, harm is mitigated.

REFERENCES

1. Campos, B., Pickering, A.C., Rocha, L.S. et al. Diversity and pathogenesis of Staphylococcus aureus from bovine mastitis: current understanding and future perspectives. BMC Vet Res 18, 115 (2022).
2. Cheng WN, Han SG. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. Asian-Australas J Anim Sci. 2020 Nov;33(11):1699-1713.
3. Wilke, Ga; Wardenburg, J. Role of disintegrin and metalloprotease 10 in S. aureus alpha-hemolysin mediated cellular injury. Proc Natl Acad Sci 107 (30). 13473-13478. (2010).
4. Uniprot, Q2G1X0 · HLA_STAA. SAOUHSC_01121 . Alpha Hemolysin.

ACKNOWLEDGEMENTS

Thank you to everyone who is involved with the VIP program and the resources they have provided for the successful involvement of undergraduate students into research opportunities. I would also like to thank the faculty for the time taken to mentor students through this process.