12-6-2023

Sle1 (*aaa*) and Alpha Hemolysin (*hla*) *Staphylococcus aureus* Antigens as a Potential Vaccine for Cows

Roz Al Janabi
Boise State University

Madeline Clark
Boise State University

Juliette Tinker
Boise State University

We acknowledge support from the Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grants #P20GM103408, P20GM109095, and 1C06RR020533. We also acknowledge support from The Biomolecular Research Center at Boise State, BSU-Biomolecular Research Center, RRID:SCR_019174, with funding from the National Science Foundation, Grants #0619793 and #0923535; the M. J. Murdock Charitable Trust; Lori and Duane Stueckle, and the Idaho State Board of Education.

Sle1 (*aaa*) and Alpha Hemolysin (*hla*) *Staphylococcus aureus* Antigens as a Potential Vaccine for Cows

Abstract

Knowledge of *Staphylococcus aureus* is essential to understanding how this pathogen causes different types of infections in humans. It can cause skin superficial infections and gastroenteritis. It can also cause infections in the joints and wounds, therefore causing humans to be severely ill by causing sepsis or infection in the blood. It is also very commonly antibiotic-resistant. In the lab, we are working with this priority pathogen because of antibiotic resistance. Cows can get *S. aureus* from humans, and it causes mastitis. This affects the dairy industry which is very important in the United States, but also specifically important in Idaho. We are making our vaccine through what's called a chimera, we are fusing antigen proteins from *S. aureus* to cholera toxin (CT). The antigens we are using is Aaa/Sle1; a peptidoglycan hydrolase and adhesin, and Hla, a hemolytic pore protein. We have performed PCR and cloned this gene into a vector for chimera expression. The importance of this research is to improve productivity and quality of life by preventing *S. aureus* in cows. Everything that we learn about developing a vaccine for a cow can also translate to developing a vaccine for humans.

Comments

We acknowledge support from the Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grants #P20GM103408, P20GM109095, and 1C06RR020533. We also acknowledge support from The Biomolecular Research Center at Boise State, BSU-Biomolecular Research Center, RRID:SCR_019174, with funding from the National Science Foundation, Grants #0619793 and #0923535; the M. J. Murdock Charitable Trust; Lori and Duane Stueckle, and the Idaho State Board of Education.

Sle1 (*aaa*) and Alpha Hemolysin (*hla*) *Staphylococcus aureus* Antigens as a Potential Vaccine for Cows

Roz Al Janabi, Madeline Clark and Dr. Juliette Tinker
Department of Biological Sciences, Boise State University, Boise, ID 83725

BOISE STATE UNIVERSITY

Abstract

Knowledge of *Staphylococcus aureus* is essential to understanding how this pathogen causes different types of infections in humans. It can cause skin superficial infections and gastroenteritis. It can also cause infections in the joints and wounds, therefore causing humans to be severely ill by causing sepsis or infection in the blood. It is also very commonly antibiotic-resistant. In the lab, we are working with this priority pathogen because of antibiotic resistance. Cows can get *S. aureus* from humans, and it causes mastitis. This affects the dairy industry which is very important in the United States, but also specifically important in Idaho. We are making our vaccine through what's called a chimera, we are fusing antigen proteins from *S. aureus* to cholera toxin (CT). The antigens we are using is Aaa/Sle1; a peptidoglycan hydrolase and adhesin, and Hla, a hemolytic pore protein. We have performed PCR and cloned this gene into a vector for chimera expression. The importance of this research is to improve productivity and quality of life by preventing *S. aureus* in cows. Everything that we learn about developing a vaccine for a cow can also translate to developing a vaccine for humans.

Background

Peptidoglycan hydrolase (Sle/Aaa) is involved in the splitting of the septum during cell division. Binds to both alpha and beta chains of human fibrinogen as well as fibronectin, which suggests a role in the colonization of host factor-coated material or host tissue. Also exhibits lytic activity against *S.carnosus* and *S. aureus* cells. Hydrolyzes the link between N-acetylmuramoyl residues and L-amino acid residues during cell division. The gene *aaa* encodes Sle and I is in the secreted cell Surface (cell wall).

Alpha hemolysin (Hla) is known for its cytotoxic properties. When exposed to cell walls (particularly red blood cells), 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 cell, resulting in lysis. This is especially dangerous in RBC's and is one of the most harmful proteins of *S. aureus*.

Objective

This study is to characterize the antigens *aaa* and *hla* as vaccine components. These *S. aureus* antigens were found based on the expression of these genes in cow's milk. Bovine mastitis is an inflammation of the mammary gland caused by an infection of *S. aureus*. It leads to decreased milk production and loss of profit for farmers. By cloning *different S. aureus* genes, we can use them to help in the production of an *S. aureus* vaccine.

Workflow

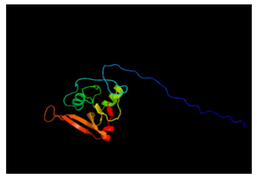


Figure 1. Phyre 2 Protein Folding Structure

PCR amplifications of *S. aureus* virulence factor to isolate *aaa* gene. Created and ran an agarose gel electrophoresis of the PCR products to determine the DNA fragment length. The length of the fragments was: Upper Band PCR – about 400 bps (size of *aaa* PCR). Lower Bands: primer dimers.

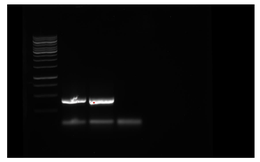


Figure 2. Size of *aaa* PCR – upper band, 400 bps

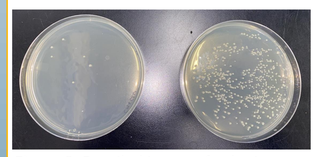


Figure 3. Detailed image of colonies on ampicillin plates

Conducted *E. coli* transformation with ligation mix containing the *aaa* gene. Incubated cells at 42°C, plated on ampicillin-containing media, and observed colonies.

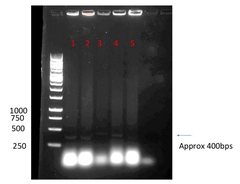


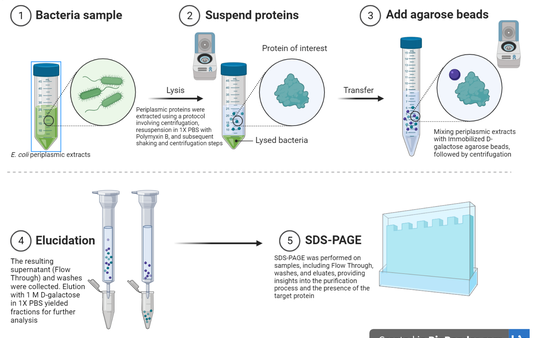
Figure 4. Obtained a band for each colony in colony PCR of *E. coli* transformants, this suggests that the PCR reaction was successful in amplifying the target DNA sequence present in each of the transformed colonies

Glycerol Stock

Positive *E. coli* clones (Clone 6 and Clone 8) were identified using colony PCR and gel electrophoresis. For glycerol stock preparation, swabs of these clones were resuspended in LB broth with 20% glycerol and 0.5% glucose. The resulting mixture was pipetted into two tubes, one for each clone, and frozen at -80°C

Protein Purification and SDS-PAGE

The protein purification process aimed to isolate the target protein (CT-chimeras) from *E. coli* periplasmic extracts.



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

Figure 5. Hla protein structure

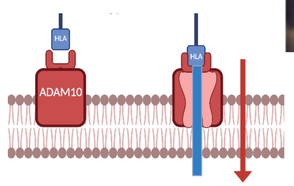


Figure 8. Representation of how hla binds to ADAM10 receptor on cell membranes and drills a pore through the wall. Created by Biorender.

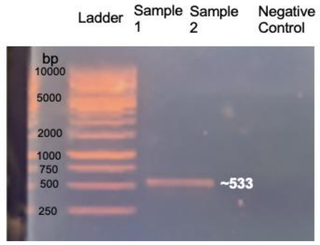


Figure 6. PCR of hla: 533 bps. Shows clear indication of successful hla replication. Successful sample 1 PCR next to a 10000 bp ladder.

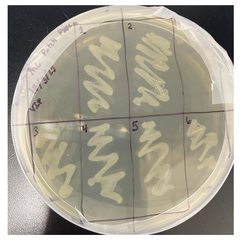


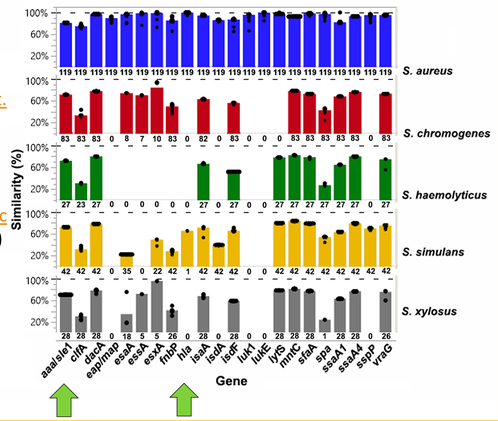
Figure 7. After transforming hla into the E. coli plasmid, a patch plate was performed to see if the E. coli was viable. Next step would be to plate the transformed E. coli on ampicillin plate.

Additional 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.

Bioinformatics

Gene Name	Gene Symbol	Protein Description[1]	Location[2]	Adhesin probability[3]	Helices	MHC I	MHC II	ML score	Homology to human?
aaa/sle1	SAOUHSC_00427	autolysin and adhesin	cell wall	0.905	0	18	8	93.1	No
hla	SAOUHSC_01121	alpha toxin; host cell lysis	extracellular	0.636	1	16	4	98.5	No

Bioinformatics was performed using Vaxign (https://violinet.org/vaxign/) to determine antigen score and NCBI (https://www.ncbi.nlm.nih.gov/) to determine prevalence and homology of antigens



Results and Discussion

Confirmation of Gene Integration

The initial step involved conducting Colony PCR to confirm the successful integration of the *aaa* and *hla* gene into *E. coli* colonies. Selected colonies from patch plates exhibited positive results, showing the amplification of a specific 600-bp fragment (*aaa* gene), and 530 bp fragment (*hla* gene). Gel electrophoresis further validated these findings, revealing distinct bands corresponding to the expected size.

Preservation of Positive Clones

Positive *E. coli* clones, validated through Colony PCR, were chosen for the preparation of glycerol stocks. These stocks were stored at -80°C, ensuring the prolonged viability and accessibility of the selected clones for future experiments. The corresponding plasmids, carrying the *aaa* and *hla* gene, were successfully logged with researcher's name, establishing a comprehensive record of the genetic material introduced into the *E. coli* strains.

Specific Protein Purification - Affinity Chromatography

Affinity chromatography involved the binding of the *aaa* and *hla* protein to immobilized D-galactose agarose beads. This step demonstrated the selective interaction of the target protein with the affinity matrix, laying the foundation for obtaining a purified fraction of the *aaa* and *hla* protein with high specificity.

SDS-PAGE Analysis for Purity Assessment

Following affinity chromatography, eluates underwent SDS-PAGE analysis.

Next steps and outlook

These studies indicate that we can successfully clone the Hla and Sle1 genes into a vector for expression in E.coli. It remains unclear if cholera toxin A2/B chimeras can be purified. Cholera toxin will help to stimulate an immune response to the vaccine, but it only does that for certain vaccines, we don't completely understand how it works. The next direction in the lab is the comprehensive analysis of purified Aaa and Hla protein through SDS-PAGE. This gel electrophoresis technique will allow for the assessment of protein purity, molecular weight determination, and the identification of any contaminants. The results from SDS-PAGE will provide important validation of the success of the protein purification process.

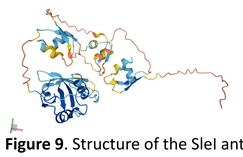


Figure 9. Structure of the Sle1 antigen

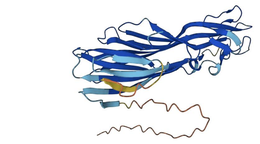


Figure 11: Structure of Hla antigen

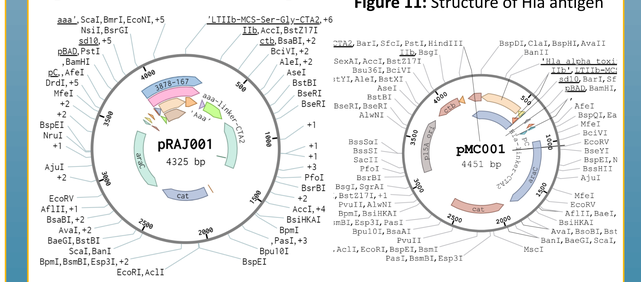


Figure 10. Design of Plasmids pRAJ001 and pMC001 for expression of Sle1-CTA2/B and Hla-CTA2/B chimeras

Acknowledgements:

Thanks to Haley Bridgewater, Noah Souza, Matthew Tamayo and Annie Walgren for technical and bioinformatics support.

We acknowledge support from the Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grants #P20GM103408, P20GM109095, and 1C06RR020533. We also acknowledge support from The Biomolecular Research Center at Boise State, BSU-Biomolecular Research Center, RRID:SCR_019174, with funding from the National Science Foundation, Grants #0619793 and #0923535; the M. J. Murdock Charitable Trust; Lori and Duane Stueckle, and the Idaho State Board of Education.