Prophage induction from *Staphylococcus aureus* bovine mastitis isolates

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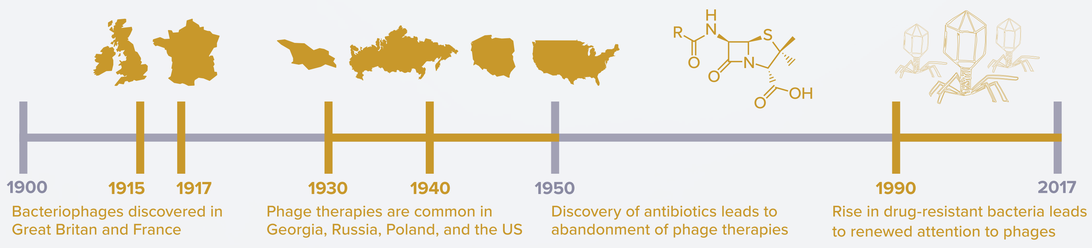
ABSTRACT

Mastitis is a major concern to the dairy industry as it is the main cause of economic losses to producers due to reduced milk production, low milk quality, and costs of animal treatment. The use of antibiotics to treat bovine mastitis has raised concerns over the development of antibiotic resistant bacteria, antibiotic residues in milk and milk quality. Furthermore, *Staphylococcus aureus* is the most common cause of mastitis and is of particular concern due to low cure rates following treatment. Research into non-antibiotic treatments for *S. aureus* mastitis is a critical need. Bacteriophages and endolysins have the ability to meet this need. Therefore, the process of phage induction was investigated as a means to isolate and identify novel bacteriophage specific to *S. aureus*. Twenty-two *S. aureus* bovine mastitis field isolates were obtained from bulk tank milk samples originating from dairy farms in Central California. Pulsed-Field Gel Electrophoresis was performed on these isolates and it was determined there were 15 genetically distinct isolates. Prophage induction was accomplished by exposure to Ultra Violet light. Optical Density and colony-forming units (CFU) were tracked for 5 time points following exposure to UV light. Lysates from each time point were spot tested onto the control strain *S. aureus* ATCC 19685 as well as the 15 field isolates. Nine bacteriophage were isolated from this process and two *S. aureus* field isolates were found to be more susceptible to bacteriophage attack than *S. aureus* 19685. Prophage induction offers the potential to quickly screen, identify and characterize bacteriophage and their endolysins for use in non-antibiotic therapies of mastitis.

INTRODUCTION



Frederick W. Twort in Great Britain (1915) and Félix d'Hérelle in France (1917) independently discovered bacteriophages. D'Hérelle coined the term bacteriophage, meaning "bacteria eater," to describe the agent's bacteriocidal ability. Soon after making their discovery, Twort and d'Hérelle began to use phages in treating human bacterial diseases such as bubonic plague and cholera.[1] During the 1930's and 40's, phage therapies were commonly used in Georgia, Russia, Poland and the US. Interest in these treatments declined after the discovery of antibiotics in the 1940's and further study of phage therapies was virtually abandoned. However, with the rise of drug-resistant bacteria in the 1990's, the therapeutic potential of phages has received renewed attention.

Additionally, finding alternatives to antibiotics used in animal health and food safety has brought increased interest in applications of bacteriophages and their products for use in agriculture. *Staphylococcus aureus* is a major concern in mastitis in dairy cattle and small ruminants, with estimated losses of $2 billion annually.[2] On the average farm in the US, at least 30% of cows will have clinical or subclinical *S. aureus* mastitis.[3] Therefore, there is great potential for developing non-antibiotic therapies for mastitis as well as on-farm bio-control agents. Isolating bacteriophages often requires a cumbersome process of screening sewage and environmental samples. Here we outline a method by passing traditional modes of screening to quickly identify and isolate prophages from *S. aureus* field isolates for further characterization.

METHODS

Bulk tank milk samples originating from dairies in the Central Valley of California were screened for *S. aureus* on 5% washed bovine blood agar (BBA). Bacterial isolates were confirmed as *S. aureus* by Beta-hemolysis on blood agar, gram-stain, Catalase test, KOH String test, and Coagulase test. Isolates were passed 3 times on BBA for isolation and stored in Brain Heart Infusion (BHI) broth with 20% glycerol at -80C. Pulsed Field Gel Electrophoresis (PFGE) was performed according to the CDC protocol for typing *S. aureus*.[4] Bacteriophage Induction: An overnight culture of *S. aureus* isolates was used to inoculate a fresh culture and incubated at 37C for 3 hours. Optical Density (OD) was adjusted to .3 – .4 at 600 nm. Cultures were exposed to UV light for 30 seconds with gently rocking. Following UV light exposure, 5 ml of 2x BHI broth was added to each culture and incubated at 37C for 1 hour. A time point sample was taken each hour for 5 hours following exposure, OD at 600nm, Colony Forming Units (CFU) and Plaque Forming Units (PFU) was measured at each time point. Each time point sample was centrifuged at 1000 g for 10 minutes and lysates were passed through a .2 um filter. Spot test and plaque assays were performed with a *S. aureus* phage cured field isolate.

RESULTS

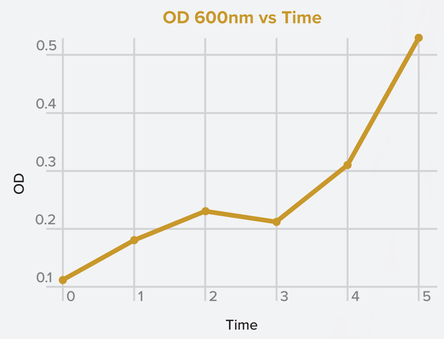


Figure 1: Optical Density following UV light exposure of field isolate.

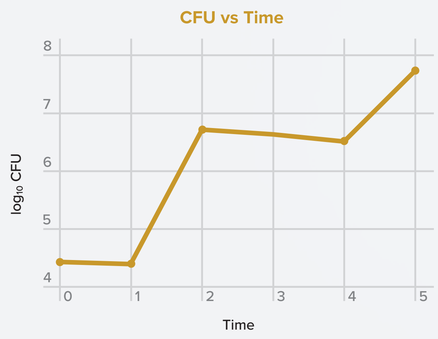


Figure 2: Colony Forming Units following UV light exposure of field isolate.

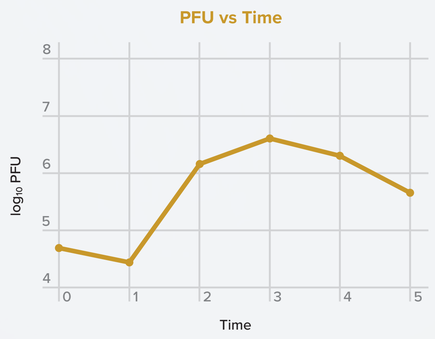


Figure 3: Plaque Forming Units following UV light exposure of field isolate.

Figure 4: Plaque Assay of prophage isolate.

Screening of twenty-two *S. aureus* bovine mastitis field isolates obtained from bulk tank milk samples from dairy farms in Central California were confirmed as *S. aureus* by Beta-hemolysis on blood agar, gram-positive, Catalase positive, KOH String test negative, and Coagulase positive. Molecular typing of Twenty-two *S. aureus* isolates was performed by PFGE and confirmed fifteen genetically unique *S. aureus* field isolates. Prophage induction by UV light revealed the change in OD and CFU over the 5 time points consistent with prophage induction. Spot Tests were performed with lysates of the 15 UV light exposed field isolates. Nine of the fifteen *S. aureus* field isolates harbored prophages capable of lysogeny and bacterial lysis. Two of the *S. aureus* field isolates were more susceptible to phage attack than indicator strain *S. aureus* ATCC 19685. Plaque Assays of nine lysates show change of PFU over the five time points consistent with prophage induction. Figure 1 through Figure 3 are representative of the nine bacteriophage induction time point curves.

CONCLUSION

Here we outline a process to quickly identify and isolate prophages from *S. aureus* field isolates. Nine out of 15 *S. aureus* field isolates harbored prophages capable of lysing susceptible host strains of *S. aureus*. We also show more than half of the *S. aureus* field isolates harbor prophages and can be used as a source for discovering novel phages and endolysins. Further study of the lytic capability of these prophage isolates is required as well as investigation of genes encoding endolysins. The use of prophage induction to identify novel phages and endolysins has the potential to decrease time and resources and increase the rate discovery in this field.

Bacteriophages and endolysins have the potential to replace antimicrobial therapy in lactating and dry cows. Development of phage-derived products for use as therapeutic and prophylactic treatments in humans and animals offers an opportunity for alternative treatments to antibiotics. Furthermore, phage-derived products have applications in agricultural use and food safety. Currently, there are FDA approved phage products on the market in the US, for use in food safety and bio-control and there is ongoing research of phage products for uses in environmental decontamination, plant pathogens and bio-preservation of foods.[5] Further research of bacteriophages and endolysins as sources of novel antimicrobials is a critical need as the increase in antibiotic-resistant bacteria has become a major public health concern.

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