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 uses 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 (PFGE) was performed on these isolates and it was determined there were 15 genetically distinct isolates. Prophage induction was accomplished by exposure to UV Ultraviolet light. Optical Density and colony-forming units (CFU) were tracked for 5 time points following exposure to UV light. Lysis rates from each time point were spot tested onto the control strain S. aureus ATCC 6538P 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 80/86. Prophage induction offers the potential to quickly screen, identify and characterize bacteriophage and their endolysins for use in non-antibiotic therapies of mastitis.

### 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 uses 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 (PFGE) 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. Lysis rates from each time point were spot tested onto the control strain S. aureus ATCC 6851 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 80/86. Prophage induction offers the potential to quickly screen, identify and characterize bacteriophage and their endolysins for use in non-antibiotic therapies of mastitis.

### METHODS

Bulk tank milk samples originating from dairies in the Central Valley of California were screened for S. aureus on 5% tryptic bovine blood agar (BBA). Bacterial isolates were confirmed as S. aureus by Beta-hemolysis on blood agar, gram-positive, Catalase test, KOH String test, and Coagulase test. Isolates were exposed to UV light for 30 seconds with gently rocking. Following UV light exposure, 3 UV colonies were picked to each culture and incubated at 37°C 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, 3 UV colonies were picked to each culture and incubated at 37°C for 3 hours. A time point sample was taken each hour for 3 hours following exposure. OD at 600nm, Colony Forming Units (CFU) and Plaque Forming Units (PFU) were measured at each time point. Each time point sample was centrifuged at 1000 g for 10 minutes and lysates were passed through a 0.2 nm filter. Spot test and plaque assays were performed with a S. aureus phage cured field isolate.

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.