Investigating alternative methods such as bacteriophages and bacteriocins to control mastitis organisms

(PRJ-0092-2016)

University of KwaZulu-Natal

Quarter 2 2016 (April 2016 till June 2016)

Project goals

Goal 1 - Obtain Staphylococcus aureus strains of interest representative of the interior of the country from Dr Martin van der Leek.

Achievements

Achieved.

No Non-achievements / underperformance has been reported

Goal 2 - Isolate bacterial strains of interest from clinically infected dairy cows from the KwaZulu-Natal region and provide Dr van der Leek with these.

Achievements

Bacterial strains of interest (Staphylococcus spp., Streptococcus spp., Escherichia coli) have been collected and stored at UKZN. However, these cultures have not been sent to Dr van der Leek.

Non-achievements / underperformance

Isolated bacterial cultures from the KZN region have not been sent to Dr van der Leek.

Reasons for non-achievements / underperformance

We are awaiting feedback from Dr van der Leek regarding the this transfer of cultures.

Planned remedies for non-achievements / underperformance

Dr van der Leek will notify the UKZN research team once his research team are ready to process the KZN-region cultures. At this juncture, we

shall courier the isoalted cultures to him. Until such time, the bacterial cultures are all being kept in storage.

Goal 3 - Isolate and classify phages active against the S. aureus strains from (1) and (2). The same milk samples used for isolation of bacterial strains will be used for isolation of phages.

Achievements

Achieved. To date, we have a growing phage bank with approximately 220 staphylococcal phages in storage. These phages are actively lytic against both the UKZN and UP staphylococcal strains. New pahges are constantly being added to the pahge bank.

No Non-achievements / underperformance has been reported

Goal 4 - Isolate bacteriocins from Staphylococcal and Streptococcal strains, and coagulase-negative Staphylococcus spp. from raw milk. Futhermore, isolate bacteriocins from Bacillus spp.

Achievements

Bacteriocin extraction for staphylococcal strains has taken place.

Non-achievements / underperformance

Bacteriocin extraction for streptococcal and coagulase-negative staphylococcal strains, Bacillus spp. has not been achieved.

Reasons for non-achievements / underperformance

A unanimous decision was made by the research leaders that bacteriocin extraction and screening for bacterial cultures other than the staphylococci be re-scheduled for 2017. This was primarily due to lack of manpower to carry out the extractions and screenings, coupled with order of priority in terms of the disease most prevalent in dairies presently, i.e., *Staphylococcus aureus*-induced mastitis.

Planned remedies for non-achievements / underperformance

Extractions and screenings are scheduled to take place from January 2017 onwards. At this point, Mxolisi Ndlela, the MSc student

currently working on *Staphylococcus aureus*-induced mastitis, will have completed the staphylococcal studies and will move onto the other species.

Goal 5 - Run in vitro screening of the phages and bacteriocins to investigate their efficacy and required lethal doses against S. aureus, before proceeding with in vivo trials in Years 2 and 3.

Achievements

In vitro screening has been completed for phages. However, bacteriocin screening is only envisaged to take place in July 2016 during the *in vivo* trial off-season. It is envisaged that bacteriocins will be incorporated into *in vivo* trials in 2017, and not during the trials scheduled to take place in 2016. .

The third *in vivo* trial using phages has been completed and data analysis and interpretation is currently underway.

Non-achievements / underperformance

Phages and bacteriocins have not been screened together either *in vitro* or *in vivo*, for combined activity effects.

Reasons for non-achievements / underperformance

A decision was made between principal investigators that bacteriocin research be included in the study as a secondary to phage research in these initial stages of the project. This is due to the time that it would have taken to run the *in vitro* bacteriocin screening, which would have delayed progression of the *in vivo t*rials that are currently underway. Hence, bacteriocin+phage screening in vitro will take place in 2017.

Planned remedies for non-achievements / underperformance

The final decision was that the initial rounds of *in vivo* trials proceed using the most effective phages only. Thereafter, bacteriocin extraction and in vitro analysis was scheduled to take place from May-August 2016. This will be followed by subsequent inclusion of bacteriocins in *in vivo* studies in a new set of trials scheduled to begin in Year 2017.

Goal 6 - Optimise protocols for large-scale production of phages and bacteriocins, in vitro, for use in vivo in Years 2 and 3.

Achievements

Optimisation of phage production protocols to satisfy the requirements for *in vivo* trials for up to 30 experimental cows has been successfully carried out.

Bacteriocin upscaling is only envisaged to take place from January 2017, in preparation for bacteriocin inclusion into *in vivo* trials starting in March/April 2017.

Non-achievements / underperformance

Protocols for large-scale production of bacteriocins has not been undertaken.

Reasons for non-achievements / underperformance

It was decided upon during project meetings that bacteriocin research be included in the study as a secondary adjunct to the phage research in these initial stages of the project.

This is due to the time that it would have taken to run the *in vitro* bacteriocin screening, which would have delayed progression of the *in vivo t*rials that are currently underway.

Planned remedies for non-achievements / underperformance

Bacteriocin extraction, *in vitro* screening and production optimization will take place from January-March 2017.

This will be followed by subsequent inclusion of bacteriocins in *in vivo* studies in new trials scheduled to start in April 2017.

Goal 7 - In addition to S. aureus, isolate strains of Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, and Escherichia coli from both the KwaZulu-Natal region as well from the interior (Dr van der Leek) and isolate phages and test bacteriocins against these pathogens.

Achievements

Strains of *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis,* and *Escherichia coli* from the KwaZulu-Natal region have been undergoing with a total of 74 different strains in storage. Strains from the interior (Dr van der Leek) will be obtained between February-March 2016. The isolation and testing of phages against the staphylococcal strains has been completed. We are currently running antibiotic resistance assays on these bacterial strains.

Non-achievements / underperformance

Phages and bacteriocins have not been isolated and screened against streptococcal species and *E. coli*. This will take place Year 2017. This will be carried out only once the primary *in vivo* trials testing phage and bacteriocin activity against staphylococcus-induced mastitis has been completed.

Reasons for non-

achievements / underperformance

Project meetings lead to the decision that staphylococcus-induced mastitis was to be the priority for these initial stages of the project in Years 2015 and 2016. It was decided that only once we get concrete proof-of-concept data that is applied in large-scale trials, can we move onto control of the other problematic microbes. These additional studies will be initiated from January 2017.

Planned remedies for non-achievements / underperformance

Isolation and screening of phages and bacteriocins against the greater array of mastitis-causing microbes will take place from January 2017. This will only be once the large-scale *in vivo* trials testing phages against staphylococcus-induced mastitis have been completed.

Goal 8 - Explore alternative diagnostic methods for the detection of mastitis in raw milk, i.e., methods that differ from SCC alone.

Achievements

The following key outputs have been acheived:

1. Continuation of calibration of the near infrared instrument using optimized reference methods, in order to obtain predictive models which will enable the NIR instrument to be used to predict milk fats, lactose and proteins in milk.

2. Milk inoculated with *S. aureus* isolate (in 5 replicates) together with a control were analyzed using gas chromatography for VOC's profiles and data is being analysed. VOC profiles for coagulase negative staphylococci, Streptococcus agalactiae, Streptococcus uberis, and Escherichia coli are currently being analysed.

3. Polymerase chain reaction has been conducted on bacterial isolates, the two unidentified bacillus species isolated from pasteurized milk samples and the isolates from University of Pretoria. The amplicons will be sent to Stellenbosch DNA sequencing facility to obtain the sequence of each of these isolates 16S rDNA in order to definitively classify differernt strains between specific types species.

Non-achievements / underperformance

None. All research is on track and progressing optimally.

Reasons for non-achievements / underperformance

Not appliable.

Planned remedies for non-achievements / underperformance

Not applicable.

Income and expenditure statement

Income and expenditure statement	Income and Expenditure Apr-Jun2016.pdf
Unnecessary spending during period	No

Popular Report

No file has been uploaded

Additional documentation

Mdu Progress Report_20.06.2016.doc

Statement

Levy funds were applied only for the purposes stated in the contract	Yes
Levy funds were applied in an appropriate and accountable manner	Yes
Sufficient management and internal control systems were in place to adequately control the project and accurately account for the project expenditure	Yes
The information provided in the report is correct	Yes