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

(PRJ-0092-2016)

University of KwaZulu-Natal

Quarter 3 2016 (July 2016 till September 2016)

Project goals

Goal 1 - Test susceptibility of the Staphylococcus aureus and Streptococcus spp. strains from the interior of the country (from Martin van der Leek - MvdL) to the phages that are being assessed in this study: Jan - July 2016

Achievements

The cultures received from Dr M vd Leek have been screened, *in vitro*, for susceptibility towards the phages currently being used at UKZN. These are phages that have been used in extensive *in vitro* analyses and *in vivo* trials. All *S. aureus* strains screened showed susceptibility towards phage lysis by the three primary phages currently in use. The streptococcal phages were screened against only tow streptococcal phages. These bacterial strains demonstrated susceptibility towards phage lysis. The next phase is to screen a larger number of bacterial strains from MvdL against the wider range of phages that we have stored in the UKZN phage bank (for both staphylcocci and streptococci). We also must genetically characterise each of the strains to ensure that we are not working with duplicate strains.

No Non-achievements / underperformance has been reported

Goal 2 - If the said bacterial strains are not susceptible against the strains from MvdL, then isolate and classify new phages active against these bacterial strains: Jan - Jul 2016

Achievements

To date, we have only documented susceptibility of all bacterial from MvdL to the phages being screened, hence the need for isolation of new phages has not arisen. However, it is highly likely that we will encounter strain tolerance as we process further bacterial strains from MvdL.

No Non-achievements / underperformance has been reported

Goal 3 - Isolate bacteriocins from staphylococcal and streptococcal strains and coagulase-negative staphylococci from raw milk. Furthermore,

isolate bacteriocins from Bacillus spp.

Achievements

Bacteriocin extraction for staphylococcal strains has taken place.

Non-achievements / underperformance

Bacteriocin extraction for streptococci and coagulase-negative staphylococci strains has not been achieved.
Bacteriocin extraction from *Bacillus* spp. has also not been acheived.

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 Year 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 South African 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 4 - Run in vitro screening of the phages and bacteriocins to investigate their combined compatibility and required lethal doses against S. aureus (both from KZN and MvdL): Apr - Aug 2016

Achievements

In vitro screening has been completed for phages. However, bacteriocin screening is only envisaged to take place from January 2017. 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.

Non-achievements / underperformance

Phage:bacteriocin compatibility screening has not yet taken place.

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* trials that are currently underway. Hence, bacteriocin+phage screening *in vitro* will take place in Year 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 which was scheduled to take place from May- August 201 will now be postponed until January 2017. 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 5 - Optimise protocols for large-scale production of phage and bacteriocins, in vitro, for use in vivo trials (Year 3): May - Sep 2016

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 March 2017, in preparation for bacteriocin inclusion into *in vivo* trials starting in April/May 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

trials 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/May 2017.

Goal 6 - In addition to S. aureus, scout for and screen phages and bacteriocins active against strains of Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis and Escherchia coli from both the KwaZulu-Natal region as well as from MvdL. It is envisaged that in vivo trials against the streptococci take place in Year 3: Jan - Dec 2016

Achievements

Strain isolation and storage of *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Escherichia coli* from the KwaZulu-Natal region has been ongoing with a total of 74 different strains in storage. The first batch of strains from the interior (Dr van der Leek) have been received. The isolation and testing of phages against the staphylococcal strains has been completed. Antibiotic resistance assays on these bacterial strains has also been undertaken.

No Non-achievements / underperformance has been reported

Goal 7 - Microscopic and molecular analysis of phages used in in vivo trials: Jan - Aug 2016

Achievements

Molecular analysis of phages using partial genome sequencing has been ongoing. Results are yet to be anlaysed, however preliminary data shows strain differences.

Non-achievements / underperformance

Microscopic analysis of new phage isolates has not been achieved to date.

Reasons for non-achievements / underperformance

The transmission electron microscope that is required for morphology studies on the phages has been out of commission for several months. It is currently being repaired and we hope to have

access to the machine by the middle of November 2016.

Planned remedies for non-achievements / underperformance

If the equipment remains unrepaaired in November, then we will have to outsource the microscopy work to another institution, preferably within the province.

Goal 8 - Explore alternative diagnostic methods for the detection of mastitis in raw milk, i.e., methods that differ from SCC alone: Jan - Oct 2016

Achievements

Studies have shown a general increase in electrical conductivity (EC) and decrease in pH across milk samples inoculated with *Staphylococcus aureus.* This was demonstrated by a decrease in the pH to values less than 5.5, with EC above 6.5 mS/cm for both uninoculated milk and 1x10e2 cfu/ml inoculated milk. A standard overnight stock of S. aureus inoculated into fresh milk caused a rapid increase in electrical conductivity and a rapid decrease in pH in all milk samples.

Non-achievements / underperformance

Near infrared analysis (NIRA) is still outstanding.

Reasons for non-achievements / underperformance

We are in the process of calibrating the NIRA unit with raw milk samples across the spectrum of infection, i.e., highly infected to uninfected.

Planned remedies for non-achievements / underperformance

NIRA is currently underway and is being used to estimate milk composition (fats, lactose and proteins) to distinguish between infected and non-infected milk samples and to identify *Staphylococcus aureus* associated volatile metabolite profile. Identification of *S. aureus* volatile metabolites profile will enable us to distinguish *S. aureus*-induced mastitis cases from other pathogens as volatile metabolites profile amongst mastitis causal pathogens differ. NIRA will be complete by 26 October 2016.

Goal 9 - Complete second block of in vivo trial testing phages against S.

aureus-induced bovine mastitis. (NB. In vivo trials in Year 3 of the study will include screening of both phages and bacteriocins as a combined treatment): Jan - Nov 2016

Achievements

Second block of trials has been completed and results have been analysed (see attached a preliminary report).

No Non-achievements / underperformance has been reported

Goal 10 - Data analysis and interpretation, publications, conference presentation (local and international), and popular articles and presentations: Apr 2016 - Feb 2017

Achievements

I.Basdew and M.Ndlela attended an international mastitis conference in Nantes, France, 7-9 September 2016. This conference was hosted by the IDF. Both candidates delivered talks on their respective research and both talks were very well received with delegates showing an ardent interest in the use of phages for mastitis control.

M.Ndlela is currently completing an application to upgrade his MSc study on the in vivo application of pahges for mastitis control to a PhD study, starting from Year 2017. M.Shinga is currently writing research chapters and has completed Chapters 1 (Literature Review) and Chapter 2 pH and EC changes in milk).

No Non-achievements / underperformance has been reported

Income and expenditure statement

Income and expenditure statement	I&E report.pdf
Unnecessary spending during period	No

Popular Report

No file has been uploaded

Additional documentation

Prelim Second Trial Report.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