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

(PRJ-0062-2015)

University of KwaZulu-Natal -

Quarter 3 2015 (July 2015 till September 2015)

Project goals

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

Achievements

Dr van der Leek and his team have just started culture collections & antibiograms from mastitic milk samples submitted to the Milk Lab at Onderstepoort. Their emphasis is on the routine testing of herds for high SCC & the bulk of those isolates have already been tested for sensitivity. Once they have collated their cultures, then we will engage in the exchange. Dr Inge-Marie Petzer is on sabbatical until the end of the year, so progress has been slower in this regard.

Furthermore, Dr Petzer would also like assurance that our cooperation will result in outputs for which we both can claim credit, i.e. likely publications. Dr van der Leek and UKZN are compiling an agreement to this effect to state that all parties will benefit from mutual co-operation in the way of scientific (or other) publications that arise from the collaborative efforts.

No Non-achievements / underperformance has been reporterd

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

This has not been achieved as yet.

Non-achievements / underperformance

Dr van der Leek has not yet been provided with cultures.

Reasons for non-achievements / underperformance

Dr van der Leek and his team have just started culture collections & antibiograms from mastitic milk samples submitted to the Milk Lab at

Onderstepoort. Their emphasis is on the routine testing of herds for high SCC & the bulk of those isolates have already been tested for sensitivity. Once they have collated their cultures, then we will engage in the exchange. Dr Inge-Marie Petzer is on sabbatical until the end of the year, so progress has been slower in this regard. Dr van der Leek has suggested that we keep our cultures in storage and delay the exchanges until they have fully collated their cultures.

Planned remedies for non-achievements / underperformance

Once Dr van der Leek's team have collated their cultures, then we will engage in the exchange. Dr Inge-Marie Petzer is on sabbatical until the end of the year, so progress has been slower in this regard. Dr van der Leek has suggested that we keep our cultures in storage and delay the exchanges until they have fully collated their cultures.

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

We have successfully isolated new phages for the various *Staphylococcus aureus* strains isolated in Goal (2) above. We have isolated a total of 115 phages, specific to *S. aureus*. These phages are currently being stored until further use.

Non-achievements / underperformance

No phages have been isolated with respect to Goal (1).

Reasons for non-achievements / underperformance

Cultures have not been exhcnaged between parties as yet. Dr Inge-Marie Petzer is on sabbatical until the end of the year, so progress has been slower in this regard. Dr van der Leek has suggested that we keep our cultures in storage and delay the exchanges until they have fully collated their cultures.

Planned remedies for non-achievements / underperformance

Dr van der Leek and his team have just started culture collections & antibiograms from mastitic milk samples submitted to the Milk Lab at Onderstepoort. Their emphasis is on the routine testing of herds for high SCC & the bulk of those isolates have already been tested for sensitivity. Once they have collated their cultures, then we will engage in the exchange.

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

Achievements

Protocol development and acquisiton of reagents required for bacteriocin isolation have been ongoing. The research team are preparing materials in order to begin bacteriocin isolation in November 2015. We will begin with isolation from the streptococci and staphylococci first. Isolation of bacteriocins from coagulase-negative staphylococci and *Bacillus* spp. is planned for February-March 2016.

Non-achievements / underperformance

Bacteriocins have not yet been isolated from any bacterial strains of interest.

Reasons for non-achievements / underperformance

The MSc student undertaking the bactericin rseearch has developed the protocols and has begun acquiring the materials necessary in order to being the isolations. However, there have been many delays in acquiring chemicals and reagents required for the protocols. These reagents are only just beginning to be delivered to us.

Furthermore, the student has been busy preparing for field trials testing the phages against mastitic cows.

Planned remedies for non-achievements / underperformance

Reagents have been ordered and protocols developed in order to begin with bacteriocin isolation in November 2015. During this first phase of isolation, bacteriocins will be extracted from the staphylococci and streptococci only. Isolation from coagulase-negative staphylococci and *Bacillus* spp. will take place in Jan-Feb 2016.

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

The isolation and *in vitro* screening of new phages active against *S. aureus* is underway and has been ongoing since April 2015. Typical assays include lethal dose, phage titer and multiplicity of infection. These tests are imperative in order to assess whether or not a phage is viable as a biocontrol agent. In terms of the specific phages that will be used durig the *in vivo* trials, the research team will be applying the phages isolated and screened during the PhD study of I.Basdew. These phages have been re-screened to confirm their activity. Phages were being produced in bulk in preparation for the field trials that started in October 2015.

Non-achievements / underperformance

Bacteriocin extraction and purification has not yet taken place.

Reasons for non-achievements / underperformance

Discussions with the MilkSA management team lead to a decision to postpone bacteriocin screening *in vivo* until Year 2 of the study, as significant exploratory work regarding its *in vitro* efficacy and compatibility with phages still needs to be determined. However, phage *in vivo* studies will be undertaken in Year 1 due to results from previous research.

Planned remedies for non-achievements / underperformance

Bacteriocin extraction and purification protocols have been compiled. Reagents required for the extraction and processing is in the process of being acquired. It is envisaged that bacteriocins will be extracted and stored during the last quarter of Year 1 (2015). These bacteriocins will be screened *in vitro* in January-February 2016, before incorporation into *in vivo* trials with phage treatments (September 2016 onwards).

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

Achievements

The large-scale propagation of phages for use during *in vivo* trials is constantly uderway as we need to be ready for a field trial as soon as we have enough infected animals - according to Animal Ethics regulations, we cannot artificially inoculate an animal and therefore have to wait until infection sets in on its own in specific herds. Wecurrently have enough phage to run two large-

Non-achievements / underperformance

The large-scale production of bacteriocins for *in vivo* use has not been initiated.

sclae trials and are still continuously increasing production *in vitro*.

Reasons for non-achievements / underperformance

Although bacteriocin extraction protocols have been compiled, discussions with the MilkSA management team lead to a decision to postpone bacteriocin screening *in vivo* until Year 2 of the study. It was recognised that significant exploratory work regarding its *in vitro* efficacy and compatibility with phages still needs to be determined. Based on these *in vitro* studies, we can determine how to best incorporate the bacteriocins into a trial in a supplementary fashion to the phages.

Planned remedies for non-achievements / underperformance

Year 1 of the study will be allocated towards bacteriocin extraction, purification, bioassays and phage-bacteriocin compatibility tests. Large-scale production will take place in Year 2 of the study, at which time bacteriocin will be applied with the phages in the field trial.

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

To date, 15 strains of *Streptococcus agalactiae*, 11 strains of *Streptococcus dysgalactiae*, 13 strains of *Streptococcus uberis*, 12 strains of *Escherichia coli* and 8 strains of coagulase-negative staphylococci (to be confirmed as coagulase-negative) have been isolated and stored. These strains are all from the KwaZulu-Natal region. Phages have been isolated against the streptococcal strains.

Non-achievements / underperformance

Strains from the interior from Dr van der Leek have not been received. Furthermore, the extraction of bacteriocins active against the streptococci, *E. coli* and coagulase-negative staphylococci has not yet taken place. Identification of phages lytic towards the coagulase-negative staphylococci has not yet taken place.

Reasons for non-achievements /

underperformance

The first half of the year had been allocated towards preparing *S. aureus* phages for field trials. Hence, limited time resources have been allocated towards the identification, isolation and screening of phages and bacteriocins against the streptococci, *E. coli* and coagulase negative staphylococci.

Planned remedies for non-achievements / underperformance

Full-scale *in vitro* screening of phages active against the streptococci, and isolation and *in vitro* assays for *E. coli* and coagulase-negative staphylococci will be conducted from Jan-March 2016. Screening includes lethal dose assays, phage titers and phage compatibility tests. Bacteriocin extraction and screening is due to being in November 2015. We will begin by isolating and screening bacteriocins active against *S. aureus* and the streptococci. Bacteriocins active against *E. coli* and the coagulase negative staphylococci will be isolated and screened from Jan-March 2016.

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

Achievements

The student that has been working on this project has performed satisfactorily. Key achievements include:
1. Optimisation of the suitable ratio of milk sample to ethanol to 1:25 (60µL of milk sample to 1500µL of ethanol), absolute ethanol is required to separate milk fats from the proteins by cold precipitating proteins at -20°C that would otherwise interfere with milk fat UV absorbance
2. Investigating the optimum incubation time required for protein precipitation by absolute ethanol. The aim was to evaluate whether or not longer incubation time would precipitate more proteins thus far less interference than when these when milk fats are measured in UV region. We found that 1 hour was the ideal incubation time required for protein cold precipitation.
3. Since the UV absorbance optima of total fats/lipids is between 202-215nm in the UV region, however, the optimum UV absorbance shifts with change in concentration of fat/lipids, such that solutions with higher fat content will have an UV optima at a higher wavelength than solutions with lower fat content. This problem however can be solved by evaluating all the possible wavelengths in the UV region of 202-215 and determining the suitable wavelength, by choosing a wavelength that has better correlation (upon linear fit on the standard curve) between points representative of solutions used. Forcato et al., 2005 found best correlation at a wavelength of 208nm, so we investigated and found that 205nm is best suitable, discrepancies

may primarily be due to different re-agent grades, milk samples used or instrument for measuring the absorbance.Therefore we have successfully produced a milk fat standard curve using prepared standard milk samples of milk fat content between 10-150 mg/mL, ratio between absolute ethanol and milk sample, 1:25, and 1 hour incubation time required for cold protein precipitation and absorbance was measured at 205 nm in a UV instrument. The standard curve had a correlation of 0.991 between points. Using this standard curve we have estimated the milk fat concentration of milk test samples from the following dairies; Hulley-Oldfield (10 samples), Schiever (5 samples) and A/5/04-0003 Black (10 samples).

No Non-achievements / underperformance has been reporterd

Income and expenditure statement

Income and expenditure statement	Detail_CC_Report_UIB7.pdf
Unnecessary spending during period	No

Popular Report

No file has been uploaded

Additional documentation

No file has been uploaded

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