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

(PRJ-0092-2016)

University of KwaZulu-Natal

Quarter 4 2016 (October 2016 till December 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

Completed. *Staphylococcus aureus* and *Streptococcus* spp. strains showed susceptibility to the three major phages (SaPh1, SaPh2, SaPh3) being assessed in the current project. These bacterial strains also demonstrated resistance to 8 of the 11 most commonly applied antibiotics used for the management of bovine mastitis. We would now like to undertake studies on a further batch of microbes from Dr van der Leek's laboratory.

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

Not applicable as the bacterial strains received from Dr van der Leek were susceptible to phages SaPh1, SaPh2 and SaPh3. We would however, like to pursue further assessments on a next batch of microbes from Dr van der Leek's laboratory in order to assess any changes in microbial susceptibility/resistance to said phages.

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

Bacteriocins have been sucessfully isolated from *Staphylococcus aureus*, *Streptococcus galactiae*, *Strep. dysgalactiae*, *Bacillus* sp., and coagulase-negative staphylococci. These bacteriocins have been kept in storage.

No Non-achievements / underperformance has been reported

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 testing phages and bacteriocins against the staphylococcal strains from KZN and MvdL has been carried out. Plate assays were carried out to assess bacterial susceptibility to the control agents. Results showed that phages appear to impose superior control over the pathogen in comparison to the bacteriocin only. We plan to now run combination-screening assays where staphylococci will be exposed to phages+bacteriocins combined in a single suspension.

No Non-achievements / underperformance has been reported

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

Achieved. We have adopted the method of liquid-bulk fermentation to grow phages in large enough quantities for our field trials. This involves inoculating an overnight culture of S. aureus with phages SaPh1, SaPh2 and SaPh3 and incubating this suspension for 12hr. This is then followed by purification of the phage:bacterium suspension and final centrifugation of the bacterium-free suspension in order to obtain a pure virus pellet. This pellet is then reconstituted in phage buffer.

No Non-achievements / underperformance has been reported

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

Phages and bacteriocins for the *Streptococcus uberis*, *Strep. galactiae* and *Strep. dysgalactiae* have been isolated and stored. This was carried out for bacterial cultures from both MvdL and KZN.

Non-achievements / underperformance

Phages and bacteriocins for *Escherichia coli* have not been isolated.

Reasons for non-achievements / underperformance

The major causal pathogen for bovine mastitis are the staphylococci and streptococci. Hence emphasis was placed on disease management where these microbes are involved.

Planned remedies for non-achievements / underperformance

Phage and bacteriocin scouting for *E. coli* will take place in the latter part of Year 2017, only after the *in vivo* trials testing phage/bacteriocin combinations against *Staphyloccus aureus* and *Streptococcus* spp. has been completed.

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

Achievements

Molecular analysis of phages used in the study has been completed.

Non-achievements / underperformance

Microscopic analysis has not be completed.

Reasons for non-achievements / underperformance

The microscopy facility at UKZN has not been fully operational in the past 6 months. This is mainly due to continuous malfunctioning of the transmission electron microscope, which is the primary utility for the identification and observation of phage structure and infection process.

Planned remedies for non-achievements / underperformance

We have been assured that the transmission electron microscope will be repaired and fully functional by week 2 of January 2017. We hope to then be able to document the structural and biological features of the said phages.

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

Gas chromatography-mass spectrophotometry was, in the last quarter of 2016, used to distinguish between volatile organic compound profiles in milk inoculated with the different mastitis-causing microbes. Results showed that GC-MS did not provide distinctive enough VOC profiles to enable rapid differentiation between species, and hence rapid detection of mastitic organisms specifically.

Non-achievements / underperformance

Results showed that GC-MS did not provide distinctive enough VOC profiles to enable rapid differentiation between species, and hence rapid detection of mastitic organisms specifically.

Reasons for non-achievements / underperformance

Due to the wide scope of similarity in the VOC's produced by both the beneficial and pathogenic microbes, distinguishing between VOC's specific to disease-causing bacteria was not very accurate. Furthermore, the differential VOC's produced by the pathgenic microbes are in levels that are too low to be detected using GC-MS.

Planned remedies for non-achievements / underperformance

It is envisaged that the application of NIRA will detect even microsopic levels of VOC's in milk and will provide a level of accuracy in terms of distinguishing pathogenic mcirobes in milk that supercedes that of GC-MS. NIRA calibrations and screening have been completed. We are in the process of collating and analysing data.

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

Completed. We have planned to run a third block of trials using a larger number of test animals. This trial is envisaged to take place in Quarter 1 of Year 2017.

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

Data analysis is currently underway for all the research completed in the latter part of Year 2016. This is specifically applicable to Mxolisi Ndlela (Phages and mastitis) and Mduduzi Shinga (Diagnosis of Mastitis). Papers can only be prepared once all data ahs been analysed and compiled.

No Non-achievements / underperformance has been reported

Income and expenditure statement

Income and expenditure statement	Inc & Exp Q4_MilkSA.pdf
Unnecessary spending during period	No

Popular Report

Q4-Popular Report.doc

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