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

(PRJ-0092-2016)

University of KwaZulu-Natal

Year 2016 (January 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

Staphylococcus aureus and *Streptococcal* spp. have been received and assessed. Staphylococcal trains have been screened for antibiotic resistance and for susceptibility towards the phages used in the animal trials, and *Streptococcal* spp. have been screened for antibiotic resistance. It was found that 9 out of the 10 staphylococci exhibited antibiotic resistance and all stpahylococci showed susceptibility towards the phages. Two of the 5 streptococci showed antibiotic resistance.

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

All the staphylococci from MvdL demonstrated susceptibility towards the phage attack. However, the streptococci were not subjected to phage susceptibility screening. Phages were isolated against the streptococci but *in vitro* bioasssays will be carried out in 2017.

Furthermore, we have generated a phage bank where lytic phages isolated from raw milk are routinely screened against 5 staphylococcal strains and then stored for later use (particularly in the case of inactivity of the current phages brought about as a result of bacterial resistance).

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 successfully isolated from the said species. These have been stored for futher use in 2017.

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 has been carried out for staphyloccci only. 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.

Bacteriocin extraction and screening for the streptococci will be carried out in 2017.

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

Large-scale (not commerical scale) production of phage cocktails for field trials has been achieved. We have adopted the method of liquid-bulk fermentation to grow phages in large enough quantities for *in vivo* trials. This involves inoculating a 12hr culture of *S. aureus* with phages SaPh1, SaPh2 and SaPh3 and incubating this suspension for a further 12hr. This is then followed by purification of the
phage:bacterium suspension by removal of bacterial residue and cells that may have not lysed, and final centrifugation of the bacterium-free suspension in order to obtain a pure virus pellet (translucent, honey-coloured, gelatinous pellet). This pellet is then reconstituted in phage buffer through 2hr shaking at 4°C, and stored at 4°C until required.

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 have not been isolated for *Escherichia coli.* Furthermore, *in vivo* trials screening the streptoccoal phages and bacteriocins has not be undertaken.

Reasons for non-achievements / underperformance

1. *Escherichia coli* and its phages: *E.coli* is a common place microbe present in the dairy environment but in the context of the current study, was not the primary organism being targeted for phage therapy. While studies have shown that antibiotic resistance does exist in *E. coli* in the dairy environment, it was considered prudent to first understand the mechanisms of phage treatment (+bacteriocin) in an *in vivo* system using *Staphylococcus aureus* as the starting organism.

2. *Streptococcus* spp. *in vivo* trials: These trials have not been undertaken due to time constraints. Furthermore, we require naturally infested animals, i.e., animals diagnosed with clinical mastitis where the streptococci are the causal pathogens. We have yet to source enough animals diagnosed with streptococcal-mastitis.

Planned remedies for non-achievements / underperformance

1. *E.coli* studies: Both *in vitro* and *in vivo* studies on *E. coli* will follow in the third quarter current year of study: Year 2017.

2. Streptococci studies: *In vivo* trials for streptococcus-induced mastitis will hopefully also take place on the third quarter of the current year. This is a highly feasible option as we have had feedback from our local dairy collaborator (from KwaZulu-Natal Midlands) that he has recently noted an increase in the incidence of streptococcus-induced mastitis in his herds.

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

Achievements

Molecular analysis of phages has been completed and is hence an achieved goal. The microscopic analysis of these phages has been partially completed, in terms of structure and infection process.

Non-achievements / underperformance

The full infection cycle of the phage has not been morphologically documented.

Reasons for non-achievements / underperformance

We have been experiencing ongoing problems with the transmission elelctron microscope based at UKZN.

Planned remedies for non-achievements / underperformance

The TEM based at UZKN has become operational again, as of late December 2016. We are now in a position to document the specific stages of infection by the 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 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.

Analysis of data generated during NIRA is currently underway. It is envisaged that the data from this will show specific correlation patterns to specific bacteria, based on the volatiles they produce during their metabolism.

No Non-achievements / underperformance has been reported

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 trialsm 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 in the latter stages of completion for all pf the research completed in both Years 2015 and 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 has been analysed and compiled.

No Non-achievements / underperformance has been reported

Income and expenditure statement

Income and expenditure statement	Income & Exp_annual_27.1.17.pdf Report_Diagnostic protocols.doc Report_In vivo.doc
Unnecessary spending during period	No

Popular Report

Popular Report.doc
Report_Diagnostic protocols.doc
Report_In vivo.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