Abstracts of Plenary and International Keynote Speakers

SOUTH AFRICAN SOCIETY FOR MICROBIOLOGY

“GREEN LIVING THROUGH SCIENCE AND TECHNOLOGY”

SASM 2016
### Sunday 17th January 2016

**VENUE: AFRICAN SKY**

**17:30 – 17:45**

**Welcome and Introduction**
- Prof S Singh - Chair of the Organizing Committee
- Prof J Albertyn - President of South African Society for Microbiology (SASM)

**17:45 - 18:45**

**PLENARY SESSION - CHAIR: PROF SUREN SINGH**

**PL1:** Dr Karen E. Nelson - President of the J. Craig Venter Institute (JCVI)
The human microbiome and implications for health and disease

19:00 – Late
Welcome Cocktail and Beer Fest

### Monday 18th January 2016

**VENUE: AFRICAN FIRE 1-3**

**08:20-09:00**

**Opening Ceremony**
- Prof S Singh - Chair of the Organizing Committee
- Prof J Albertyn - President of South African Society for Microbiology (SASM)
- Prof A Bawa – Vice Chancellor (Durban University of Technology)
- Prof D Pillay - Deputy Chief Executive Officer (RISA) at National Research Foundation (NRF)

**PLENARY SESSION - CHAIR: PROF J ALBERTYN**

**PL2:** Prof Russell Terrence Hill - Director of the Institute of Marine and Environmental Technology. Professor at University of Maryland Centre for Environmental Science.
Bacterial symbionts in marine sponges: key in nutrient cycling in coral reef ecosystems

**09:45-10:30**

**PL3:** Prof Holger Jenke-Kodama - Assistant Professor at the Okinawa Institute of Science and Technology Graduate University (OIST), Okinawa, Japan.
The biological role of palytoxin: Really a toxin or a modulator of microbial communities?

**10:30-11:00**

**Poster session 1**

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<td><strong>VENUE: AFRICAN FIRE 1</strong></td>
<td><strong>VENUE: AFRICAN FIRE 5-7</strong></td>
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<tr>
<td><strong>Environmental Biotechnology</strong></td>
<td><strong>Enzyme Technology</strong></td>
<td><strong>Molecular Biology</strong></td>
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<tr>
<td>Chair: Dr Hafizah Chenia</td>
<td>Chair: Dr Feroz M Swalaha</td>
<td>Chair: Prof Florian F. Bauer</td>
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<tr>
<td>165 Amplicon sequencing: Unravelling microbial diversity from environmental samples</td>
<td>Overexpression of native <em>Saccharomyces cerevisiae</em> SNARE genes increased heterologous cellulase secretion</td>
<td>Development of a high throughput cell-free metagenomic screening platform</td>
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<td>Prof Ashwani Kumar</td>
<td>Dr Riaan den Haan</td>
<td>Prof Marla Trindade</td>
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<td>Dr Harisingh Gour Central University, Sagar, India.</td>
<td>University of the Western Cape</td>
<td>University of the Western Cape</td>
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<tr>
<td>Biodegradation of 2,4-dichlorophenol by bacteria indigenous to contaminated groundwater and activated sludge samples</td>
<td>Cutinolytic activity from the phytopathogen <em>Pseudomonas syringae pv. maculicola</em></td>
<td>Differential gene expression of the <em>mtp</em> gene in <em>M. tuberculosis</em> complex clustering strains: an insight into the hypoxic response in planktonic and biofilm cultures</td>
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<tr>
<td>Ms Boitumelo Setlhare</td>
<td>Dr Matsobane Tlou</td>
<td>Mrs Natasha Naidoo</td>
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<tr>
<td>University of KwaZulu-Natal</td>
<td>University of Johannesburg</td>
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<th>11:45-12:00</th>
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<tr>
<td>Assessing microbial activity on pyrite concentrate mineral surface during bioleaching</td>
<td>Design of smart hydrogels for use as support matrices for immobilisation of cellulases in saccharification of lignocellulose</td>
<td>Development of a cassava mosaic disease (CMD) resistant cassava through enhanced post-transcriptional gene silencing</td>
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<tr>
<td>Mr Didi Xhanti Makaula</td>
<td>Mr Vuthari Lovemore Mahlale</td>
<td>Ms Helen Walsh</td>
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<td>University of Cape Town</td>
<td>University of Limpopo</td>
<td>University of the Witwatersrand</td>
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<td>Time</td>
<td>Session 4</td>
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| 12:00-12:15| S1.04 Desulphurization flotation for the selective removal of pyrite from coal discards using microorganisms  
Mr Winfull Msipa  
University of Cape Town | S2.04 Non-productive binding of cellulases onto lignocellulosic hydrolysate inhibitors  
Mr Sizwe Mhlonogo  
University of Stellenbosch | S3.04 Development and optimisation of real-time polymerase chain reaction assays coupled with high-resolution melt curve analyses for the detection of Cryptosporidium parvum and Ascaris lumbricoides  
Mrs Marile Lombard  
Stellenbosch University |
| 12:15-12:30| S1.05 Identifying the primary source tracking markers for the detection of faecal contamination in harvested rainwater  
Ms Monique Waso  
Stellenbosch University | S2.05 Screening for acetyl xylan esterases from a hot desert metagenome  
Ms Fiyinfoluwa Adesioye  
University of Pretoria | S3.05 Minimum inhibitory concentration determination of quaternary ammonium compounds in bacteria harbouring QAC resistance genes  
Ms Marisa Coetzee  
University of the Free State |
| 12:30-12:45| S1.06 Analysis of microbial species within tank bioleaching systems  
Dr Robert Huddy  
University of Cape Town | S2.06 Comparing the biodegradation of bisphenol-a using fungal and bacterial laccases  
Mr Alaric Prins  
Cape Peninsula University of Technology | S3.06 Sequences enhancing cassava mosaic disease symptoms are associated with South African cassava mosaic virus infection  
Prof Chrissie Rey  
University of the Witwatersrand |
| 12:45-13:00| S1.07 A search for potential bacterial bioindicators of heavy metal contamination in the upper crocodile river  
Mr Cornelius Mahlanza  
University of Pretoria/Agricultural Research Council | S2.07 An evaluation of the effects on the non-catalytic galactomannan binding ability of β-mannosidases on their activity and synergism with a mannase during galactomannan hydrolysis  
Ms Mariska Thoresen  
Rhodes University | S3.07 Integrated genomics, RNA-seq transcriptomics and biochemical network analysis of Kluyveromyces marxianus reveals genetic programming  
Mr Du Toit Schabort  
University of the Free State |
| 13:00-14:00| LUNCH                                                                  |                                                                          |                                                                          |
| 14:00-14:30| S4.01 Stimulating the development of the South African bioeconomy sector through the CSIR biomanufacturing industry development centre  
Dr Daniel Visser  
CSIR Biosciences | S5.01 Enzymes bridge agricultural output to industrial products  
Prof Zheng Xiang Wang  
Tianjin University of Science & Technology | S6.01 Treatment options in a post antibiotic era  
Prof Robert Bragg  
University of the Free State |
| 14:30-14:45| S4.02 Consolidated bioprocessing for the hydrolysis and fermentation of raw starch  
Mrs Rosemary Cripwell  
University of Stellenbosch | S5.02 Comparison of two closed-coupled solar pasteurization systems for the treatment of roof-harvested rainwater  
Mr Brandon Reyneke  
Stellenbosch University | S6.02 Listeria monocytogenes and Salmonella typhimurium effects on CACO-2 cells pretreated with bioengineered probiotic Lactobacillus casei expressing listeria adhesion protein in simulated intestinal fluid  
Dr Mapitsi Thantsha  
University of Pretoria |
| 14:45-15:00| S4.03 Effect of non-regulated pH on the dynamics of dark fermentative biohydrogen production with suspended and immobilized cell culture systems  
Ms Joelle Penniston  
University of KwaZulu-Natal | S5.03 Physiological responses of carboxyphilic microalgae to elevated carbon regimes  
Ms Virthie Bhola  
Durban University of Technology | S6.03 Cocktails of probiotics pre-adapted to multiple stress factors are more robust under simulated gastrointestinal conditions than their parental counterparts and exhibit enhanced antagonistic capabilities against Escherichia coli and Staphylococcus aureus  
Dr Moloko Mathipa  
University of Pretoria |
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Presenter</th>
<th>Institution</th>
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<tbody>
<tr>
<td>15:00-15:15</td>
<td>S4.O4</td>
<td>Filamentous fungi as an alternative source of safe natural pigments</td>
<td>Ms Caryn Hobbs</td>
<td>University of Cape Town</td>
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<td>S5.O4</td>
<td>Isolation and identification of polycyclic aromatic hydrocarbon - degrading bacteria in artificially polluted soil</td>
<td>Ms Rosina Makofane</td>
<td>Agricultural Research Council</td>
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<td>S6.O4</td>
<td>Coexistence of free-living amoebae and bacteria in selected water distribution systems of Johannesburg hospitals</td>
<td>Mr Petros Muchesa</td>
<td>University of Johannesburg</td>
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<tr>
<td>15:15-15:30</td>
<td>S4.O5</td>
<td>Engineering bacteriophage resistance in <em>Geobacillus thermoglucosidasius</em> towards a robust platform for biofuels production</td>
<td>Mrs Leonardo van Zyl</td>
<td>University of the Western Cape</td>
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<tr>
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<td>S5.O5</td>
<td>Polycyclic aromatic hydrocarbon-degrading bacteria isolates as potential soil fertility enhancers</td>
<td>Ms Maryam Bello-Akinosho</td>
<td>Agricultural Research Council/ University of Pretoria</td>
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<tr>
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<td>S6.O5</td>
<td>Changes in nasopharyngeal microbiota profiles preceding and at the onset of pneumonia in South African infants: a pilot study</td>
<td>Mrs Shantelle Claasen</td>
<td>University of Cape Town</td>
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<tr>
<td>15:30-15:45</td>
<td>S4.O6</td>
<td>Enzymatic saccharification of pre-treated banana pseudostem and fermentation of the hydrolysate to ethanol</td>
<td>Mr Lesetja Legodi</td>
<td>University of Limpopo</td>
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<td>S5.O6</td>
<td><em>In vitro</em> assessment of <em>Bacillus</em> isolates as a potential probiotic feed additive for <em>Tilapia</em></td>
<td>Ms Yourisha Pillay</td>
<td>Durban University of Technology</td>
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<td>S6.O6</td>
<td>Assessment of Human Immunodeficiency Virus Type-1 Subtype C drug resistance mutations in North West Province, South Africa</td>
<td>Ms Lorato Modise</td>
<td>North West University</td>
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<tr>
<td>15:45-16:00</td>
<td>S4.O7</td>
<td>Cloning, expression and characterization of cyanate hydratase from the thermophilic fungus <em>Thermomyces lanuginosus</em> SSBP</td>
<td>Mr Bibhuti Ranjan</td>
<td>Durban University of Technology</td>
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<td>S5.O7</td>
<td>Isolation and bioactivity characterization of endophytic bacteria from indigenous <em>Kigelia africana</em></td>
<td>Ms Zanele Nkosi</td>
<td>University of KwaZulu-Natal</td>
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<td>S6.O7</td>
<td>Simultaneous enhancement of phenolic compound degradation by <em>Acinetobacter</em> strain V2 via a step-wise continuous acclimation process</td>
<td>Dr Vikas Sharma</td>
<td>University of KwaZulu Natal</td>
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<tr>
<td>16:00-16:15</td>
<td>S4.O8</td>
<td>Lab-scale assessment and adaptation of wastewater for cultivation of microalgal biomass for biodiesel production</td>
<td>Mr Luveshan Ramanna</td>
<td>Durban University of Technology</td>
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<td>S5.O8</td>
<td>Utilization of shrimp shell waste for the recovery of bioactive compounds</td>
<td>Ms Nosihle Dlamini</td>
<td>Durban University of Technology</td>
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<td>S6.O8</td>
<td>Analysis of tropane alkaloids in field grown, plant cell, and hairy root cultures of <em>Datura stramonium</em></td>
<td>Ms Farnaaz Ally</td>
<td>Durban University of Technology</td>
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<td>16:15-16:45</td>
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<td><strong>Poster session 2</strong></td>
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**Tuesday 19th January 2016**

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<tr>
<td>07:30-08:30</td>
<td>Registration</td>
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<td>08:30-09:15</td>
<td><strong>PLENARY SESSION - CHAIR: PROF KUGEN PERMAUL</strong></td>
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<td><strong>PL4: Prof Ashok Pandey - Deputy Director CSIR's National Institute for Interdisciplinary Science and Technology, Trivandrum.</strong></td>
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<td>Production of second generation biofuels from renewable alternative feedstocks</td>
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<td>09:15-10:00</td>
<td><strong>PL5: Prof Liang Tong - Chair of the Department of Biological Sciences Columbia University</strong></td>
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<td>Structure and function of metabolic enzymes</td>
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<th>Session 7</th>
<th>VENUE: AFRICAN FIRE 3-4</th>
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<tr>
<td><strong>Session</strong></td>
<td>Agrobiotechnology</td>
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<td><strong>Chair:</strong></td>
<td>Dr Heinrich Volschenk</td>
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<td><strong>07:01</strong></td>
<td>The role of FLO-genes in controlling population dynamics in microbial ecosystems</td>
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<td><strong>07:02</strong></td>
<td>Proteomic characterization of wine yeasts for the expression of arginases involved in urea formation during fermentation</td>
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<td>Prof Florian F. Bauer Stellenbosch University</td>
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<td><strong>10:00-10:30</strong></td>
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<td><strong>07:03</strong></td>
<td>Effect of non-saccharomyces yeast and lactic acid bacteria interactions on malolactic fermentation and wine flavour</td>
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<td>Prof Heinrich du Plessis Agricultural Research Council</td>
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<td><strong>10:30-10:45</strong></td>
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<td><strong>07:04</strong></td>
<td>The effect of closantel on faecal phosphorus, calcium and magnesium in boer goats grazed at Molelwane farm, Mafikeng</td>
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<td>Ms Relebohile Ellero North West University</td>
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| **Session 8** | VENUE: AFRICAN FIRE 1                                             |
| **Session**   | Applied Microbiology                                             |
| **Chair:**    | Prof Marla Trindade                                               |
| **08:01**     | The biology of chaotropicity                                       |
| **08:02**     | Identification of secretory M. tuberculosis biomarkers using phage display |
| **08:03**     | Reduction in total protein to increase the sporulation efficiency in a Bacillus spp |
| **10:45-11:00** |                                                                 |
| **08:04**     | Epidemiological link between exposure and risk of soil transmitted helminths infection: a case for appropriate ova detection methodologies within the Gates project |
| **11:15 -11:45** |                                         |
| **Session 9** | VENUE: AFRICAN FIRE 5-7                                           |
| **Session**   | Plant Biotechnology                                               |
| **Chair:**    | Prof Robert Bragg                                                  |
| **09:01**     | Members of the genus Brachyrhizobium are root nodulating microsymbionts of indigenous and exotic legumes in South Africa |
| **09:02**     | Diversity of cultivable bacterial endophytes associated with husk, stem, seeds and leaves of Bt and non-Bt maize plants |
| **09:03**     | Performance of soil alteration index three (A13) comparing contrasting apple soil management practices |
| **11:45-12:15** |                                                  |
| **Session 10** |                                                                 |
| **Session**   | Molecular Biology                                                 |
| **Chair:**    | Dr Daniel Visser                                                   |
| **10:01**     | Enzymatic production of prebiotic xylooligosaccharides from agricultural by products |
| **10:02**     | Management of Phytophthora infestans and Verticillium in potato production - The Canadian perspective |
| **11:45-12:15** |                                                                 |
| **Session 11** |                                                                 |
| **Session**   | Plant Biotechnology                                               |
| **Chair:**    | Dr Roshini Govinden                                                |
| **11:01**     | Multiple metal tolerant bioaccumulating mine tailing bacteria isolated from North West Province, South Africa |
| **12:01**     | RAPD profiling of Bacillus spp. with biocontrol potential and their effects on mineral composition of tomato |

**Poster session 3**

**Tea/ Coffee**

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<td>Plant Biotechnology</td>
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<td><strong>Chair:</strong></td>
<td>Dr Ashira Roopnarain</td>
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<td>19:15 - 23:00</td>
<td>SASM GALA DINNER AND AWARDS EVENING HOSTED BY</td>
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## VENUE: AFRICAN FIRE 1-3

### PLENARY SESSION - CHAIR: PROF KARL RUMBOLD

#### PL6: Prof Fengyan Bai – Professor in Microbiology at the Institute of Microbiology, Chinese Academy of Sciences

**The origin and domestication of lager beer yeast**

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<td>Chair: Dr Ahmed Idris Hassen</td>
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<td>$S19.02$ Novel applications of thermostable phytase and chitinases from <em>Thermomyces lanuginosus</em></td>
<td>$S19.03$ Non-saccharomyces yeast and acetification bacteria in balsamic-styled vinegar production: a biochemical process analysis</td>
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<td>Prof Pratyoosh Shukla, Maharshi Dayanand University, Rohtak, India</td>
<td>Dr Adarsh Puri, Durban University of Technology</td>
<td>Ms Ucrecia Hutchinson, Cape Peninsula University of Technology</td>
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<td>Dr Ashira Roopnarain, Agricultural Research Council</td>
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<td>Ms Monique Smit, CSIR Biosciences</td>
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<td>$S21.03$ Antibiogram of environmental isolates of <em>Acinetobacter calcoaceticus</em></td>
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<td>$S21.04$ Investigating the quorum sensing inhibitory and anti-virulence potential of seaweed-associated bacteria</td>
<td>$S21.05$ Disentangling the drivers of taxonomic and functional community structure in the Southern ocean</td>
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<td>“Health related water microbiology – a South African and international perspective” Thor Axel Stenstrom Durban University of Technology</td>
<td>Recent advances of feed enzymes and its application Prof. Xiuyun Ye Fuzhou University</td>
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<td>Application of metagenomic analysis to characterise microbial communities involved in biological sulphate reduction for ARD bioremediation Dr Robert Huddy University of Cape Town</td>
<td>Evaluation of activatory/inhibitory effects of various substrate pre-treatment by-products and wash liquors on a mannanolytic enzyme consortium Mr Samkelo Malgas Rhodes University</td>
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<td>Cryptococcal 3-hydroxy fatty acids protect cells against amoeba phagocytosis Ms Lynda Madu University of the Free State</td>
<td>Media and physiochemical optimisation for enhanced phycocyanin production in <em>Cyanothecer</em> sp Mrs Trisha Moghany Durban University of Technology</td>
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<td>Statistical process control for improved monitoring of purification processes: a case study in the North West province Mrs Guzéne O'Reilly North-West University</td>
<td>Gas bubble formation in fermenting and non-fermenting yeasts Ms Evodia Yolander Kgotle University of the Free State</td>
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<td>The effects of antimitochondrial drugs on <em>Cryptococcus neoformans</em> and <em>Cryptococcus gattii</em> Mrs Adepemi Ogundeji University of the Free State</td>
<td>Process development for the production of a B. subtilis isolate used in bioaugmentation Ms Prisha Naicker CSIR Biosciences</td>
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<td>Statistical process control for improved monitoring of purification processes: a case study in the North West province Mrs Guzéne O'Reilly North-West University</td>
<td>Gas bubble formation in fermenting and non-fermenting yeasts Ms Evodia Yolander Kgotle University of the Free State</td>
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**CLOSING**

**LUNCH**
The human microbiome refers to the millions of microbial species that live in and on the human body. Advances in sequencing technologies and analytic approaches to microbial community analysis have enabled an in-depth characterization of the microbial communities that are associated with humans. In humans, we now recognize that these microorganisms are essential for healthy growth and development, and alterations or long-term disruptions of our normal microbial populations have been implicated in a range of conditions including colon cancer, acne, and gastrointestinal disorders. The opportunities that we are now being presented with as a result of investigating these microbial communities are enormous. It is anticipated that we will see the development of new probiotics, novel diagnostics, and new treatments that include using the microbiome as a therapeutic. Similarly, animal microbiome studies are allowing us to broaden our understanding of their microbiomes and related applications, including animal feed conversion efficiency, microbes at the animal-human interface, and implications for infectious disease control and prevention strategies. Studying healthy and diseased human populations, their microbiomes and metabolites present significant new opportunities for defining novel diagnostics and therapeutic approaches for diseases.
Sponges harbor diverse, dense microbial communities that are specific for individual sponge species and are generally stable over time. The symbiotic bacteria in sponges can comprise as much as 30% of the weight of the sponge and the sponge together with its associated microbes is best considered as a holobiont. These are ancient symbioses that provide excellent model systems for the study of complex symbioses. We have studied the roles of these sponge microbial symbionts in cycling of nutrients, in particular nitrogen and phosphorus cycling.

Most sponges are major sources of inorganic nitrogen in the very low-nutrient coral reef environment, suggesting that sponges are not nitrogen limited. We have found that diazotrophic cyanobacteria and other nitrogen-fixing bacteria contribute substantially to the fixed nitrogen in some sponge species. Symbiotic bacteria also play roles in nitrification and in removal of nitrogen through denitrification, including by the anammox process.

Cycling of phosphorus in the coral reef environment is much less understood than nitrogen cycling. We recently unexpectedly found major reservoirs of phosphorus in marine sponges as the result of polyphosphate production by symbiotic bacteria within the sponges. Phosphorus sequestration by sponge symbionts can be important in controlling the phosphorus availability in the coral reef water column. Taken together, the role of sponge symbionts in cycling of nitrogen and phosphorus provide answers to “Darwin’s Paradox” of highly productive coral ecosystems being present in the very low nutrient waters surrounding these reefs.
THE BIOLOGICAL ROLE OF PALYTOXIN: REALLY A TOXIN OR A MODULATOR OF MICROBIAL COMMUNITIES?

Holger Jenke-Kodama
Okinawa Institute of Science and Technology Graduate University (OIST)

The first report on palytoxin was published in 1971. Its name owes to the fact that it was found in a coelenterate belonging to the genus Palythoa. The compound is a very strong toxin that converts the Na/K-ATPase into a permanently open channel. Since its discovery many questions have been remained unanswered, for instance: Who is the producer? How is it biosynthesized? How does the toxin get into the animal? What is the ecological role of palytoxin?

Whilst it seems to be accepted among scientist that dinoflagellates are the source, intriguingly, all dinoflagellate cultures studied so far produce only congeners, namely ovatoxins and ostreocins, but not the original palytoxin. From the very beginning of palytoxin research, the possibility of bacteria being the producers has also been discussed. Moreover, the function of palytoxin as protection against grazers has never been proved. Like for many other marine toxins, the ecological role of palytoxin remains a mystery.

We studied the correlation between palytoxin content in Palythoa colonies in Okinawa, Japan, and the composition of the microbial communities in their gastric cavities using LC/MS and high-throughput amplicon sequencing. Furthermore, we performed cultivation experiments with and without the addition of palytoxin. We found that the presence of palytoxin can considerably change the community structure. In some cases, dramatic dominance effects of certain bacterial groups were detected. Preliminary results of the experimental cultivation studies indicated that palytoxin effects bacterial growth and community structure.

We therefore hypothesize that in spite of its seemingly clearly established “role” as a toxin, the real biological function of palytoxin might be to modulate bacterial communities. It seems worth re-examining more generally the possible ecological roles of so-called “toxins” of marine origin.
Bioethanol as a transportation fuel is an attractive alternative as it is more energy efficient than gasoline and produces less emissions. The benefits of developing biomass to ethanol technology(s) include: increased national energy security, reduction in GHG emissions, use of renewable resources, economic benefits and creation of employment and the foundation of a carbohydrate based chemical industry. However, the utilization of lignocellulosic biomass for fuel generation has not been given the sort of attention it ought to receive. Biomass requires extensive processing involving multiple steps for hydrolysis and fermentation of the raw material for producing ethanol. Feedstocks availability, pretreatment, saccharification, fermentation and ethanol recovery are the factors, which influence the production of ethanol and needs R&D efforts for overall improvement of the production economics. The sugar platform for the bioconversion of biomass to generate bioethanol is considered as the most valuable solution to the transport fuel demand.

The Centre for Biofuels at NIIST, Trivandrum, India aims to develop technologies and processes which will address the nation’s need for making fuel ethanol from the renewable resource: biomass. The Centre directs R&D activities at the major requirements of a biomass-ethanol technology, which include production of cellulases, hydrolysis of biomass, and ethanol fermentation. Viable technologies for each of these processes would contribute to the overall process development for fuel alcohol production from cheap and renewable biomass resources.

The lecture would present issues and perspectives on the availability of (lignocellulosic) feedstocks for the production bioethanol and the bioconversion process.

References
Biotin-dependent carboxylases have central roles in the metabolism of fatty acids, carbohydrates, amino acids and other compounds, and deficiencies in several of these enzymes are linked to serious diseases, especially in infants. Acetyl-CoA carboxylase (ACC) is an attractive target for drug discovery against diabetes, cancer and other diseases. Our studies have revealed diverse molecular mechanisms for the inhibition of this enzyme. Most recently, after 13 years of effort, we have determined the crystal structure of the 500 kD holoenzyme dimer of ACC (1), illuminating a novel mechanism for regulating this enzyme.

We have also shown that the pyruvate carboxylase (PC) of Listeria monocytogenes, an important human pathogen, is a target of cyclic-di-AMP (c-di-AMP), a recently identified second messenger in bacteria with important functions in bacterial growth, stress response, virulence and other processes. Our crystal structures show that the compound is bound in an allosteric pocket and suggest that c-di-AMP inhibits the enzyme by ‘freezing’ it into a single conformation (2). Elevated PC activity in L. monocytogenes (through a reduction in the levels of c-di-AMP) causes a metabolic imbalance, which is detrimental for its intracellular growth.

References
Lager-brewing at low temperature arose in 15th century Bavaria and has become the most popular technique for alcoholic beverage production in the world. The lager yeast *Saccharomyces pastorianus* is a domesticated microbe through the hybridization between an ale yeast *S. cerevisiae* and a cryotolerant wild yeast *S. eubayanus*. The latter firstly discovered from Patagonia, Argentina exhibits 99.5% genome sequence identity with the non-ale subgenome of *S. pastorianus*. Consequently, a Patagonian hypothesis for the origin of lager yeast has been proposed. Here we show that *S. eubayanus* commonly occurs in the Tibetan Plateau and adjacent high altitude regions in west China and exhibits surprisingly high genetic and phenotypic diversity. Three distinct lineages with over 6% inter-lineage sequence divergence were identified from the *S. eubayanus* strains from China based on multiple gene sequence analyses. A Tibetan population of *S. eubayanus* exhibits 99.8% genome sequence identity with the non-ale subgenome of *S. pastorianus*. Phenotypically, the Tibetan *S. eubayanus* strains are more cryophilic than the Patagonian strains. Our results suggest that *S. eubayanus* is native to Far East Asia and that the Tibetan *S. eubayanus* population is the progenitor of lager yeast. Sequence, structure and function comparison showed that the functional maltose transporter genes in lager yeast were mainly contributed from *S. cerevisiae* MTY1 and *S. eubayanus* AGT1, with some degree of variations and recombination. We are performing comparative genomic and transcriptomic analyses to reveal the contribution of *S. eubayanus* to the psychrophilic brewing nature of lager yeast.
Next-generation sequencing (NGS) technologies are revolutionary tools for studying phylogeny and taxonomy of entire microbial community from complex microbiomes or environmental samples, which are difficult or unattainable to study with capillary sequencing or PCR-based approaches. As we know that, large numbers of microorganisms (over 99%) present in some of the environments are uncultivable with existing methodologies. Pyrosequencing based on one of the 16S rRNA region out of nine (V1-V9) allowed analyses of more environmental samples, and have revealed much greater species diversity in many environments. Our research focuses on using 16S rRNA amplicon sequencing for studying bacterial communities present in the rhizosphere of selected biofuel crops (*Jatropha*, *Pongamia*, *Ricinus*, *Thevatia* spp.) and understanding their role in managing various phenomenon around plant roots including biomass improvement. 16S rRNA gene sequences have been amplified from DNA extracted from rhizosphere soil samples of different biofuel crops and sequenced by 454 technology. MG-RAST data analysis showed the comparative differences in bacterial community structure and composition among rhizosphere of different biofuel crops. The outcomes of this work will significantly enhance our understanding of the microbial diversity, dynamics and their role in improving the biomass of energy crops.

**Keywords:** Pyrosequencing; microbial diversity; rhizosphere; biofuel crops.

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In life sciences, enzyme molecules are fundamental for any living issue, e.g. substance metabolism, energy metabolism, cell duplication. On the other hand, enzyme can be act as a power “engine” to stimulate the development and application of biocatalysis and biotransformation in industrial biotechnological manufacturing, especially in converting agricultural output to industrial products. Here, the main achievement of enzyme applications in above mentioned fields are reviewed and commented, the frontiers in enzyme research are discussed.
Many of the fermentation products and other microbial metabolites valued by food scientists and industrial microbiologists are solutes/substances which exhibit chaotropic activity; i.e. they disorder biological membranes, proteins and other types of macromolecular system. Examples of chaotropic solutes are ethanol, butanol, phenol and vanillin (as well as some salts, including MgCl$_2$ and LiCl). Hydrophobic substances, which partition into the hydrophobic domains of biomacromolecular systems (log P$_{octanol\text{ }water}$ > 1.95) also disorder macromolecules and so also have a chaotropicity-mediated mode-of-action as stressors of cellular systems, and microbial systems produce both chaotropic solutes and hydrophobic stressors as secondary metabolites. Such compounds are, however, often described using the umbrella terms ‘flavour compounds’ or ‘volatile organic substances’. Many of these chaotropes and hydrophobes play key roles in the natural ecology of microbes, most notably as antimicrobials. Recent work has been carried out to clarify the meaning of, and to quantify, chaotropicity (which can arise from diverse mechanisms). Unlike kosmotropic (macromolecule-structuring) activity, chaotropicity can limit life in industrial systems and Earth’s biosphere. Understanding ways in which microbes respond to chaotropicity-induced stresses (and the associated oxidative stress), via diverse macromolecule-protection systems, is key to mitigating product-induced stresses in biofuel-, wine- and beer-fermentations as well as other industrial systems. Such responses include the production of protein-stabilization proteins, accumulation of compatible solutes, modifications of membrane structure, and increases in energy generation (as well as production of proteins involved in the removal of reactive oxygen species). Other mitigating factors include kosmotropic substances and a reduction of temperature. This said, at low temperatures (from +10 to -20°C), chaotropicity can enhance macromolecular flexibility and thereby enhance cellular metabolism and/or reduce the temperature minimum for growth. Chaotropic substances can exist is inhibitory concentrations in many locations on Earth and, indeed, on other planetary bodies, so chaotropicity has implications for habitability or terrestrial and extraterrestrial environments.
Prebiotics are non-digestible food ingredients that stimulate growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to health. They were first identified and named by Gibson and Roberfroid in 1995, involving galactooligosaccharides (GOS) and fructooligosaccharides (FOS). Recently, xylooligosaccharides (XOS) and arabin xylooligosaccharides (AXOS) produced from hemicellulose (which is roughly 20% of biomass) have been proposed as prebiotics.

Cereals are rich in xylan (the most common type of hemicellulose), and their byproducts (e.g. straw and bran) are good sources to obtain the putatively probiotic (A) XOS. To allow uptake and metabolism in probiotic gut bacteria, the polymeric xylans is fragmented to oligosaccharides (Falck et al, 2013, Immerzeel et al, 2014).

Here we present our efforts to fractionate, enrich and subsequently hydrolyse the (arabino) xylan into oligosaccharides of relevant degrees of polymerization by enzymatic methods, along with an analysis of the enzyme-substrate interactions. Obtained (A)XOS fractions were used as carbon source in growth trials using selected putative probiotic bacteria (from the genera Bifidobacterium, Lactobacillus and Weissella) and metabolites of the same (i.e. short chain fatty acids) were analysed to evaluate potential beneficial effect(s) for the host. Results from these trials will be discussed.

Acknowledgements
This work is supported by VINNOVA, Lund University: Antidiabetic Food Centre (VINN Excellence Centre) and by the Swedish research council (VR).

References
Both late blight disease and Verticillium wilt are major problems in potato production in Canada. Late blight is caused by Phytophthora infestans, whereas Verticillium wilt is a part of early dying syndrome caused by Verticillium and other soil-borne pathogens. Renewed control strategies aimed at higher efficiency and lower environmental impact are urgently required. Research carried out for a decade in our laboratories demonstrated that we can use alternative approaches to manage and control these microorganisms in the potato production systems. These approaches could reduce the use of fungicides and pesticides and ensure the protection of human health and the environment. In this speech, I will present to you our findings using proteomics, metabolomics and gene expression profiling tools about the molecular mechanisms that potato plants possess against P. infestans. I will also share with you our latest knowledge and technologies about quantifying soil microorganisms in production fields and how some of the fungicides are tracked in potatoes in order to manage and control late blight and Verticillium wilt.
Despite significant developments in improving the properties of naturally occurring biocatalysts by protein engineering and directed evolution, the search for ideal biocatalysts for industrial applications is still a hot topic in biotechnology. The majority of enzymes that are in use today have come from 0.1% of the culturable microbial diversity. Through environmental metagenomics, on the other hand, the genes encoding useful biocatalysts from both culturable and nonculturable microbes present in the environment can be recovered. We have attempted to construct metagenomic libraries from environmental metagenomes. While screening metagenomic library constructed from a soil-compost sample containing decomposing organic residues collected from the vicinity of a hot water spring, we detected a colony with a clear halo of xylan hydrolysis. When the cloned DNA fragment of 6.238 kb was sequenced, it was found to harbour a gene encoding xylanase of 1.077 kb. The gene was cloned in *Escherichia coli* BL21 (DE3), *Bacillus subtilis* and *Pichia pastoris*. The recombinant enzyme was produced and it was purified to apparent homogeneity, which is optimally active at 80°C and pH 9.0. It liberates xylooligosaccharides from xylan and agro-residues. An enzyme variant generated by site-directed mutagenesis displayed higher thermostability. The enzyme is useful in generating xylooligosaccharides from lignocellulosic agro-residues and pre-bleaching of paper pulps that lowers chlorine requirement in pulp bleaching, and thus, useful in developing an environment-friendly pulp bleaching process.
Microbial protein engineering is one of the most capable research areas due to development of various advanced technologies in computational biology. This area is emerged as decisive in biotechnology due to scope of improvement of enzymes and their substrate specific engineering and it is evident that such enzymes are significant for its use for industrial applications. Role of various techniques of enzyme screening and customized industrial screening sometimes lack the idea of substrate specific enzyme discovery. In this study, we have worked extensively for confirmed combination of enzyme screening and its substrate specificity by using various systems biology approaches in addition to using enzyme modeling tools to originate the implementation of speedy method for industrial screening. The present work highlights the comprehensive studies on some notable microbial enzymes and understanding microbial interactions. In fact, such strategies are supportive in categorizing various components and software to recognize this approach. These applications may be scrutinized further for energy efficient optimization processes in microbial biotechnology and will be supportive to understand variety of microbial interactions.

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The feed enzyme application is the fastest growing in China's industrial enzyme sector. Research and development of feed enzymes has led to versatile applications ranging from relief of resource shortages and pollution reduction to ensuring sustainable growth in the livestock industry.

While the benefits of feed enzyme applications are well known and recognized globally, actual utilization remains low. Current offerings suffer from the following challenges that prevent them from being more widely adopted: they lack physio-chemical properties for wide industrial applications; feed enzyme expression is often low; and high production costs.

To address this trend, research and development efforts have been prioritized to focus on the following: 1) high efficacy gene selection technology; 2) functional enhancement of enzyme protein at molecular level to result in better feed application; 3) feed enzyme high protein expression and production technology; and 4) building effective feed enzyme evaluation models.
KEYNOTE LECTURES

S2.01 - OVEREXPRESSSION OF NATIVE SACCHAROMYCES CEREVISIAE SNARE GENES INCREASED HETEROLOGOUS CELLULASE SECRETION.

Den Haan, R.1, Van Zyl, J.H.2, Vogel, D.1, and Van Zyl, W.H.2
1Department of Biotechnology, University of the Western Cape, Bellville 7530, South Africa.
2Department of Microbiology, Stellenbosch University, Stellenbosch 7602, South Africa

A major obstacle to using the yeast Saccharomyces cerevisiae in single-step hydrolysis and fermentation of cellulosic material for second generation bio-ethanol production is its inferior yields of secreted heterologous cellulases. In this study we have attempted to enhance heterologous protein secretion through a rational design strategy involving proteins integral to the secretion pathway. SNAREs (soluble N-ethylmaleimide-sensitive factor attachment receptor proteins) are essential components of the yeast protein trafficking machinery and are required at the majority of membrane and vesicle fusion events in the cell. We have demonstrated an increase in secretory titers for the Talaromyces emersonii Cel7A (a cellulbiohdyrolase) and the Saccharomycopsis fibuligera Cel3A (a β-glucosidase) expressed in Saccharomyces cerevisiae through single and co-overexpression of some of the ER-to-Golgi SNAREs (BOS1, BET1, SEC22 and SED5). Overexpression of SED5 yielded the biggest improvements for both of the cellulolytic reporter proteins tested, with maximum increases of 22% for the Sf-Cel3A and 68% for the Te-Cel7A. Co-overexpression of the ER-to-Golgi SNAREs yielded proportionately smaller increases for the Te-Cel7A (46%), with the Sf-Cel3A yielding no improvement. Co-overexpression of the most promising exocytic SNARE components previously identified (SSO1 for Sf-Cel3A and SNC1 for Te-Cel7A) with the most effective ER-to-Golgi SNAREs (SED5 for both Sf-Cel3A and Te-Cel7A) yielded variable results, with Sf-Cel3A improved by 130% and Te-Cel7A yielding no improvement. This study has shown that SNARE proteins fulfil an essential role within a larger cascade of secretory machinery components that could contribute significantly to future improvements to S. cerevisiae as protein production host.
Metagenomics is the benchmark for exploring the diverse metabolic potential of microorganisms directly from any environment. Although it has been fruitful, there are numerous limitations which hamper its ability to adequately tap into novel functional space, and many microbial genomes continue to remain inaccessible. This study aimed to develop an innovative ultra-high throughput solution to overcome many of the limitations associated with classic functional metagenomics. Here, uncloned metagenomic DNA from the environment was expressed and activity screened in emulsion droplets using *Escherichia coli* and *Rhodococcus erythropolis* based cell free protein synthesis, followed by selection using flow cytometry, cell sorting, whole genome amplification and sequencing to identify the expressed gene sequences. Emulsions expressing β-xylosidase activity using both *R. erythropolis* and *E. coli* based protein synthesis were selected, and two different β-xylosidase populations were identified, corresponding to the source of the cell free protein synthesis machinery (*R. erythropolis* or *E. coli*). Heterologous expression and enzyme assays of four ORFs from the *R. erythropolis*-based FACS sorted emulsions showed that these genes are expressed up to 100 fold higher in *R. erythropolis* under their native promoters compared with expression in *E. coli*. Furthermore, these ORFs could not be identified through classical functional screening of a pCCFOS library in *E. coli*. Elimination of clone library construction saves time and cost while improving efficiency and elimination of cloning bias and heterologous expression limitations. This is the first report of screening uncloned metagenomic DNA and the development of an actinomycete based cell free protein synthesis.
Bioprocessing underpins many of the technologies described within the Bio-economy Strategy. Effective bioprocess development is central to the development of technologies within the health, agricultural and industrial sector and as such it is crucial that the NSI develops strong competency throughout the bioprocess development value chain.

The Biomanufacturing Industry Development Centre (BIDC) at the CSIR provides a platform for technology development in bio and agroprocessing. The centre has developed the necessary infrastructure and skills base to facilitate translation and piloting of proof of concept technologies into sustainable market offerings.

The objectives of the BIDC are to:

• Provide affordable and readily accessible support (in the form of specialist product development competencies including infrastructure) to SMEs, HEIs and industry partners.
• Enable localisation of international technologies
• Support creation of new enterprises and new sustainable jobs
• Supports skills development

The BIDC currently supports 12 enterprises across the bio and agroprocessing fields. We are assisting these enterprises with product and process development, market penetration and pre-commercial manufacture. Additionally we train more than 20 interns and students in the facility per year and provide short courses in bio and agroprocessing. Here we will discuss the successes and challenges encountered by the BIDC over the first 2 years of operation, and the envisaged future for the centre.
S6.O1 - TREATMENT OPTIONS IN A POST ANTIBIOTIC ERA.


It is well known that there are increasing problems with antibiotic resistance, not only in poultry production, but also human medicine. There is increasing pressure to reduce or even totally stop the use of antibiotics in animal production. When this happens, the poultry sector will be faced with very serious problems.

Our research group is actively investigating various alternatives to treatment of diseases in a post antibiotic era. These alternative treatment options include improved vaccine development, improved biosecurity, and the use of bacteriophages. All three of these treatment options will be briefly reviewed.

Our main research emphasis on improved vaccine development revolves around a novel yeast based expression system. Our current research focus on this is the development of a vaccine against avian pathogenic E. coli.

Improved biosecurity has mainly been focused on the continual use of disinfectants throughout the production cycle in poultry. The use of this system has been shown to significantly reduce bacterial counts and has also been shown to reduce the impact of infectious coryza.

Our final research area is on the use of bacteriophages, or parts thereof, in the treatment of bacterial diseases. Although phages show much potential, the very high level of specificity could make the use of bacteriophages challenging. The use of expressed phage enzymes appears to be a viable alternative to making use of highly specific bacteriophages. The specificity of expressed phage enzymes is currently being tested.
Flocculation, the non-sexual adhesion of cells which leads to the formation of large groups of cells referred to as “flocs”, is an important technological property of *Saccharomyces cerevisiae* yeast strains. This property is almost entirely dependent on the expression of individual members of the FLO gene family. *S. cerevisiae* strains all indeed possess at least four functional flocculation-inducing FLO genes, *FLO1*, *FLO5*, *FLO9* and *FLO10*. These genes encode structurally related manno-proteins whose expression will induce flocculation to various degrees, while also impacting on other cell wall properties such as cell surface hydrophobicity. In natural ecosystems, and in wine production, *S. cerevisiae* competes with a large number of other yeast species whose flocculation ability has in most cases not been investigated thus far. Our data show that many of these non-*Saccharomyces* wine species are able to flocculate efficiently. More interestingly, we observed the formation of mixed species “flocs” between both flocculent and non-floculent *S. cerevisiae* strains and several of these species, a process that has not been described previously and which we refer to as “co-flocculation”.

Using strains of *S. cerevisiae* that express specific individual FLO genes, the data show that these genes impact differently on multispecies adhesion phenotypes. Association with some species was promoted by specific FLO genes, while other species were excluded from flocs, suggesting that the selective expression of different FLO genes may allow *S. cerevisiae* to “select” specific species to build exclusive microbial groupings. The FLO genes may therefore be significant drivers of ecosystem organisational patterns, and play a wider evolutionarily relevant role in allowing favourable associations between compatible yeast. Alternatively, such a function may also be linked to the recent realisation that *S. cerevisiae* can specifically suppress the growth of certain other yeast species if direct physical contact takes place. Such a role for the FLO genes may also help to explain the evolutionary persistence of a large multigene family of genes with similar function.
S9.01 - MEMBERS OF THE GENUS BRADYRHIZOBIUM ARE ROOT NODULATING MICROSYMBIONTS OF INDIGENOUS AND EXOTIC LEGUMES IN SOUTH AFRICA

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A nodulation authentication test was conducted following the Koch’s postulate experiment under glasshouse condition using rhizobial strains previously collected from economically important forage legumes. All the tested strains of rhizobia showed effectiveness in nodulating their respective legumes from which they were initially isolated with statistically significant (p = 0.05) increase in plant biomass and nodule number in comparison with the un-inoculated controls. Cross section of pink nodules observed using the Nikon Nu microscope indicated the presence of several bacteriodes distributed throughout the nodules. To elucidate their identity and phylogenetic relatedness, the strains which resulted in effective, pink nodules were selected and characterized by means of the 16S ribosomal RNA sequence analysis. The analysis confirmed that the majority of stains associated with the nodulation of the forage legumes belong to members of the genus Bradyrhizobium with a 99% identity in their 16S rRNA, while strains of Sinorhizobium and Rhizobium spp. have also been found to be involved in the nodulation of V. unguiculata, a legume of the cowpea cross inoculation group considered to be largely nodulated by Bradyrhizobium strains. The results obtained in this study provide baseline information to the South African Rhizobium Culture Collection and contribute to an understanding of the legume rhizobium symbiosis in future selection and development of effective nodulating and nitrogen fixing strains of rhizobium for forage and pasture inoculant development.

Key words: Rhizobium, Nodulation, Nitrogen fixation, Forage legumes, 16S rRNA
The quest for quality mineral resources has led to the development of many technologies that can be used to refine minerals. Biohydrometallurgy is becoming an increasingly acceptable technology worldwide because it is cheap and environmentally friendly. High levels of elements such as potassium (K) and phosphorus (P) in iron ore minerals are known to reduce the quality and price of these minerals. To deal with this problem, recent advances in mining technologies favour the use of biohydrometallurgy, which has minimal impact on the environment.

The present study investigated a potential biological method for biobeneficiation of low-grade iron ore minerals. Microbial isolates obtained from the surface of the iron ore minerals were tested for their capabilities to reduce phosphorus and potassium contents of iron ore. Their potential to lower the pH of the growth medium and solubilisation of tricalcium phosphate were used to screen them as potential mineral solubilisers. Many Bacterial and fungal isolates were successfully screened with this method and utilised in shake flask experiments using iron ore minerals as sources of K and P. The shake flask experiments revealed that all the screened isolates have potentials to produce organic acids that aided the solubilisation of the iron ore minerals. All isolates produced significant amount of gluconic acid, acetic, citric and propanoic acid. Scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) analyses also indicated extracellular polymeric substances could play a role in mineral solubilisation.
The most common mechanisms of aminoglycoside resistance is caused by three types of enzymes (i) phosphotransferases (APHs), (ii) acetyltransferases (AACs) and (iii) nucleotidyltransferases (ANTS). These are usually present on plasmids that are claimed to be ubiquitous in the environment. The aim of this study was to determine the variations of aminoglycoside resistant bacteria in surface water and sediments of the Crocodile and Marico rivers. Heterotrophic plate count bacteria were isolated from samples using nutrient agar containing 50 μg.ml⁻¹ of kanamycin. Kanamycin resistant bacterial levels were higher in the Crocodile River compared to the Marico River. Furthermore, higher levels of these resistant bacteria were detected in sediments (10³ to 10⁴ cfu/ml) compared to the bulk water (10¹ to 10³ cfu/ml). These isolates were also tested for resistance to various other aminoglycosides such as gentamicin, neomycin and streptomycin. Preliminary results indicate that a number of the bacteria were also resistant mentioned aminoglycosides. The results that will be presented will include (i) 16S rRNA gene sequence identification of selected isolates (ii) data on MIC to kanamycin (iii) PCR based data to show if the \( \text{aph}(3')-\text{IIIa} \) and \( \text{aph}(3')-\text{IIa} \) genes are the cause of the resistant phenotypes. This study will also explore whether aminoglycoside resistance caused by APHs are ubiquitous in the aquatic environment.

**Key words:** aminoglycoside, kanamycin, minimum inhibitory concentration, phosphotransferases.
S15.O1 - THE ANTIBACTERIAL ACTIVITIES OF SOME PLANT DERIVED TRITERPENES

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The increasing prevalence of multi-drug resistant bacteria has necessitated the search for new antimicrobials from alternative sources such as traditional medicinal plants. The agar well diffusion method was employed to determine the susceptibilities of four plant derived triterpenes namely, 3β-hydroxylanosta-9, 24-dien-21-oic acid (RA5), and methyl-3β-hydroxylanosta-9, 24-dien-21oate (RA3), a mixture of oleanolic acid and betulinic acid (SF1) and a mixture of 3β-acetonyloleanolic acid and 3β-acetonylbetulinic acid (SF2), at a concentration of 10 mg/ml against seven Escherichia coli, one Bacillus cereus, five Enterococcus and nine Vibrio bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined through the microbroth dilution assay. The checkerboard method was used to determine the antibiotic-triterpene interactions, while the cytosolic lactate dehydrogenase test was used to determine the membrane damaging potentials of the triterpenes. The triterpenes RA3, RA5, SF1 and SF2 had activities against 86.4%, 54.6%, 22.7% and 9.09% of the test bacteria respectively. SF1 had the lowest MIC values ranging 0.625-10 mg/ml with lower MIC values being noted against Gram negative bacteria in comparison to Gram positive bacteria; this trend was also noted among the activities of RA3 and RA5. MBC studies proved the triterpenes to be mostly bacteriostatic. The interaction studies with ciprofloxacin were mainly ranging between indifference and antagonism. RA3 alone showed minimal membrane damaging potential with the levels of cytosolic lactate dehydrogenase released ranging from 1-36%. The results hereby show the potential that the test triterpenes have as antibacterial agents, especially against the Gram negative bacteria namely E.coli and Vibrio bacteria.
β-fructofuranosidases are hydrolytic enzymes that act on sucrose to yield the products glucose and fructose. Under high substrate conditions these enzymes display fructosyltransferase activity which results in the synthesis of fructooligosaccharides (FOS). Some enzymes display higher propensities for FOS synthesis than others, with the determinants of this activity remaining unclear. The consumption of FOS produces a prebiotic effect that positively alters the composition of the colonic microflora, and as a result is linked to improved human and animal health. The increased demand for FOS has necessitated the industrial production of these nutraceuticals. In enzymatic sucrose biotransformation processes operating at high substrate loading and temperatures between 50 and 60°C, β-fructofuranosidase activity is negatively influenced by glucose product inhibition and thermal instability.

The aim of this study was therefore to engineer the *Aspergillus japonicus* β-fructofuranosidase, FopA, to improve a FOS synthesis bioprocess. A semi-rational approach, using a combined crystal structure and evolutionary-guided approach, yielded a four amino acid combination variant displaying improved specific activity and thermostability that was able to reduce the time to completion of an industrial-like FOS synthesis reaction by 26%. The positive outcome of the semi-rational approach showed that engineering loop regions in an enzyme is a feasible strategy to improve both specific activity and thermostability, most probably due to the modification of enzyme structural flexibility. A bioinformatic tool that enables the identification of β fructofuranosidases displaying high-level FOS synthesis from protein sequence alone was also developed during the study.
The reaction between cyanide and residual sulphur species, during the processing of refractory gold ores, results in a tailings effluent stream containing thiocyanate (>300 mg/L) and residual cyanide (>20 mg/L). The release of effluent water containing thiocyanate and cyanide to the environment is prohibited, necessitating on-site treatment by chemical, physical or biological means. The Activated Sludge Tailing Effluent Remediation (ASTER™) bioprocess was developed to remediate thiocyanate and cyanide contaminated effluents with the aim of enabling recycling of process water within the plant to improve the water balance and improve effluent water quality for environmental disposal. ASTER™ technology is currently employed at a number of commercial mining operations worldwide and effectively reduces the thiocyanate and cyanide concentrations to <1 mg/L. The process relies on a complex consortium of microorganisms, in suspended flocs or attached biofilm, to metabolise the thiocyanate and cyanide. The biofilm associated microbial communities represent a reservoir of microbial diversity for the continuous operation of this system. This leads to a robust commercial bioprocess capable of treating dynamic waste water effluent streams. Previous research has linked enhanced thiocyanate destruction rates to the process operating conditions and increased biomass loading. However, very little is known about the microbial community composition and metabolic potential of the thiocyanate- and cyanide-degrading microorganisms within these communities. The microbial ecology of the laboratory-scale ASTER™ process has been investigated, using 16S rRNA gene surveys and metagenomics, to provide detailed information on the diversity and composition of the abundant microbial community members. Our on-going research is focused on the drivers leading to the formation of the microbial biofilm, and the role that these sessile microbial communities play, within the current reactor configurations, to ensure efficient thiocyanate degradation. This information can be applied to inform the further optimisation of the ASTER™ process for treatment of contaminated waste waters.
Anaerobic digestion of organic wastes results in the production of biogas and a nutrient rich digestate. The biogas that is produced via this process consists of methane, carbon dioxide and minute amounts of other gases. Methane, which forms the bulk of the biogas, is combustible. Biogas can be used for the provision of heat, light and can even be converted to electricity. This is of particular significance in South Africa where a dire energy crisis is currently prevalent. Biogas technology is still in its infancy in South Africa, although recent initiatives have successfully resulted in the accelerated uptake and understanding of the technology. Unlike with other forms of renewable energy, biogas technology offers numerous advantages. One of the major advantages is waste management which is a significant problem in South Africa. The major hurdles to the implementation of biogas technology in South Africa are the cost implications, the lack of communication, lack of ownership and the negative image of the technology caused by past failures. The provision of loans, government assistance, community workshops, wide scale communication and the implementation of prefabricated digesters could impact significantly on the increased uptake of the technology in South Africa. The adoption of biogas technology in South Africa would contribute to the well-being and economic prosperity of the country as a whole.
Carbon (C) and Nitrogen (N) are important nutrients in biological wastewater treatment systems. It has been shown that when treating wastewater with a high C:N ratio, performance decreases when this ratio falls below 0.05. This can be alleviated by the addition of exogenous N.

N-fixation is the process whereby nitrogen gas is converted into ammonia by N-fixing (diazotrophic) microorganisms. It has been demonstrated that in some instances, natural microbial N-fixation increases the amount of N available in systems treating high C:N wastewater. Apart from the obvious cost and labour savings, a major advantage of a self-regulating systems over one where exogenous N is added, is that the final effluent N concentrations are low and stable.

In this study, changes in the diazotrophic bacterial community structure in sand bioreactors at the surface (0 to -5 cm) and at depth (-15 to -20 cm) were monitored before and three months after the weekly addition of synthetic winery wastewater. The changes were compared with those in a control reactor that was not amended with wastewater. Using terminal restriction fragment length polymorphism (T-RFLP) and next generation sequencing (MiSeq) of the \textit{nifH} gene, and culture dependent techniques, it was found that: (i) the addition of high C:N wastewater lead to a significant (p <0.01) change in the diazotrophic bacterial community structure at the surface of the reactors, and that (ii) the numbers of cultureable diazotrophs, especially \textit{Azotobacter} spp. also increased significantly (p < 0.01) at the surface of the reactors after amendment with synthetic winery wastewater.
Water quality criteria for the protection of human health from faecal pathogens have historically relied on the monitoring of *E. coli* or faecal coliform organisms in environmental water regimes as well as in drinking waters. *E. coli* do serve the purpose as an indicator of faecal pollution, but will at the same time have limitations and be inadequate for the protection of human health, primarily due to the poor relationship between *E. coli* and the occurrence of human pathogens in environmental samples (e.g., Payment and Locas 2011). In recognition of this limitation, in 1999 the World Health Organization (WHO) adopted a predictive risk-based framework for the mitigation and management of faecal risks associated with waterborne exposures (Fewtrell and Bartram 2001). For drinking water supplies, the use of a comprehensive risk assessment and risk management approach (termed Water Safety Planning (WSP)) that encompasses all steps in the water supply chain from catchment to consumer (WHO 2011) is recommended. This has later been expanded to further deal with multiple transmission routes and exposure scenarios through wastewater and wastewater contaminated regimes, as expressed in the Sanitation Safety Planning (SSP). (WHO 2015). In spite of the poor relationship between indicators and pathogens, the measurements of faecal indicator organisms within the risk based approach can provide valuable information related to the magnitude and variability of faecal contamination, and hence provide insight into the expected level of potential pathogen contamination. It is however essential to link occurrence and quantities, with evidences of disease and impact within the human (and animal) populations. Water, Sanitation and Hygiene is assumed to play a major role in the global disease burden and especially in Southern Africa as expressed by Prüss et al (2002) and related to a recent update 2014 on diarrhoeal disease and environment (http://www.who.int/water_sanitation_health/gbd_poor_water/en/).

In spite of the assumed and predicted evidences there is a clear gap between the scientific information from the African continent including South Africa on occurrence of pathogens and adverse chemicals in the environment and their sources and related and measured human impact. This is in spite of that we have the methodological and laboratory capabilities for detection and quantifications.

The occurrence and quantities of different pathogens in wastewater and faecal sludge are a reflection of the incidence of disease and/or carrierhip in the societies. Are we making use of this in risk predictions and as part of an early warning system?

To what extent is this used and related to antibiotics and the frequency of resistant microorganisms, emerging organic pollutants and personal care products?

Are we making use of our knowledge of the occurrence of different zoonotic organisms in the human and animal populations in the source tracking and in the relative risk prediction for downstream populations?
Are we misusing occurrence and quantities of coliforms, *E coli* or other indicators in our prediction of risks in water storage and handling, instead of using clear epidemiological follow-ups or making use of direct pathogen data or predictions?

Are we accounting for functionality and variability factors in the risk predictions for drinking water treatment, wastewater treatment and downstream impact or in disaster risk reduction and factors related to climate variability and water scarcity?

These are example of factors that need to be scrutinised and discussed when we are assessing the relevance of ongoing research related to human health, water and environmental impact.

**References**


Globally, pollution is due to a widespread use of chemicals and their release into the environment. Chlorophenolic compounds such as 2,4-dichlorophenol (2,4-DCP) are classified as priority pollutants due to their recalcitrance, persistence and toxicity. They also pose environmental and human health concerns due to their carcinogenic and mutagenic properties. The aim of this study was to isolate indigenous 2,4-DCP degrading microorganisms from activated sludge and contaminated groundwater and characterize the enzymes involved in the degradation process. Enrichment and isolation was conducted in Mineral Salt Medium (MSM), with 2,4-DCP (40 ppm) as the sole carbon and energy source. Two bacteria were isolated from contaminated groundwater sample while three isolates were obtained from the activated sludge sample. Preliminary studies showed that the bacterial isolates were able to degrade between 61.85% and 94.91% of 2,4-DCP, with a higher degradation obtained when a mixed culture of the isolates was tested. A decrease in 2,4-DCP concentration was obtained at increasing 2,4-DCP concentration, with up to 89.06, 87.42, 87.73, 77.59 and 41.24%, 2,4-DCP degradation obtained at 40 ppm, 80 ppm, 100 ppm, 200 ppm and 400 ppm, respectively for the mixed bacterial culture. Characterization and optimization of the enzymes produced by these organisms will assist in proper elucidation of the degradation pathways followed by these organisms as well as reveal their full potential for use in the abatement of sites contaminated with 2,4-DCP and other related chlorophenolic compounds.
Microbial attachment and subsequent colonization of mineral surface is a critical step in efficient mineral solubilisation and metal recovery in bioleaching operations. A better understanding of the processes and behaviour by which microorganisms attach to, and colonize, mineral surfaces may help to enhance bioleaching processes. In bioleaching, effective microbial attachment and subsequent colonisation of the mineral surface are both important factors to ensure the growth of leaching microorganisms, and therefore, enhanced mineral dissolution/bio-oxidation. In this investigation, attachment and subsequent colonization of a mixed mesophillic culture, including *Acidithiobacillus ferrooxidans*, *Leptospirillum ferriphilum*, *Acidiplasma cupricumulans* and *Acidithiobacillus caldus*, was assessed using pyrite concentrate coated glass beads. Following a period of attachment, the microbial cells colonizing the mineral surface were detached and counted, in order to determine the relative numbers of attached cells. Furthermore, the mineral surface was visualized for microbial colonization using Scanning Electron Microscope. The microbial metabolic activity of cells attached to the mineral surface was quantitatively determined using isothermal microcalorimetry (IMC). Our results indicate that a significant proportion of the microbial cells present in the inoculum attached to the pyrite surface. Microbial growth, together with an increase in redox potential, dissolution of Fe$^{2+}$ and simultaneous increase of Fe$^{3+}$ in the reactor suggested that these microorganisms were actively leaching pyrite. The increase in metabolic heat, measured using IMC, from the colonized surfaces suggested that the attached microorganisms were active and continued to leach the surface. Correlation between direct measurements of metabolites and heat generation is considered.

Quantitative assessment of microbial attachment and metabolic activity on various mineral surfaces will be discussed.
The desulphurisation of sulphide-bearing coal discards prior to their disposal has been identified as a method of preventing Acid Rock Drainage (ARD) formation. Using froth flotation, acid generating sulphide minerals such as pyrite can be removed from coal discards to obtain a low volume sulphide-rich concentrate that can be easily controlled, and a high volume benign tailings fraction that can be safely discarded. Although the technical feasibility of this process has been demonstrated, the cost of the flotation reagents used in this process is particularly high and the inorganic reagents are relatively toxic and pose a threat to the environment. Microorganisms and their metabolic products have been identified as potential agents that can be used in desulphurisation flotation. Just like conventional chemical flotation reagents, the microorganisms assist separation through surface chemical alterations that render the mineral either hydrophobic or hydrophilic thus facilitating bioflotation. In this study, the removal of pyrite from coal discards using *P. polymyxa, R. palustris, B. subtilis, B. licheniformis* and their metabolic products as bioreagents is investigated. Attachment experiments are carried out to screen for microorganisms that show a relatively higher affinity towards the pyrite or gangue minerals that are abundant in coal discards. Contact angle and zeta-potential experiments are used to study the physicochemical interactions that induce surface alterations that are crucial to the overall attachment of the microbial cells to the mineral surface. The data obtained from these experiments will inform the choice of microorganism(s) to use in bioflotation experiments.
Domestic Rainwater Harvesting (DRWH) has been proposed as a strategy to increase the volume of water available for potable and non-potable uses in rural communities and urban informal settlements. A variety of pathogens and chemical pollutants are however, present in DRWH tanks and it is imperative to identify and eliminate the primary sources of these contaminants. The aim of the current study was thus to apply microbial and chemical source tracking markers to identify the primary sources of contamination in harvested rainwater. Conventional polymerase chain reaction assays were utilised to routinely screen tank water and gutter debris for ten microbial markers. Seven chemical markers were also screened for by preparing chemical extracts as described in the US EPA method 1694. The extracts were then analysed using HPLC/MS/MS. Preliminary results indicate that adenovirus is present in 65% and 60% and *Bacteroides* HF183 in 100% and 30% of the rainwater and gutter debris samples, respectively. For the chemical markers, caffeine was detected at an average of 5.09 and 1.88 µg/L, triclosan at 1.17 and 2.43 µg/L, triclocarban at 1.06 and 2.03 µg/L and methyl paraben at 7.49 and 9.40 µg/L in the rainwater and gutter debris samples, respectively. Salicylic acid, acetaminophen and carbamazepine were all below the detection limit of 1.00 µg/L. While the DRWH tanks will continue to be monitored during high and low rainfall periods, the preliminary results suggest that anthropogenic activities may contribute to the contamination of harvested rainwater in urban areas.
Bioleaching processes, either in the form of heap leaching or mechanically-agitated tank leaching, represent approaches for efficient metal recovery from low-grade ores. Both systems rely on the metabolic activities of a consortium of aerobic microorganisms, active between pH 1 and 2, to catalyse the regeneration of ferric iron and protons through the oxidation of ferrous iron and sulfur. These acidophilic bioleaching microbes generally comprise a number of species, both bacterial and archaeal, with differing temperature optima. The efficiency of bioleaching is influenced by a number of factors including physiochemical parameters of the bioleaching environment, microbiology of the system, mineralogy, particle size and mineral liberation and the processing parameters. Assessment of the microbiology of these systems, both in terms of the composition and oxidative activity of the microbial community, is necessary to understand the effects of changes in the operating parameters on the bioleaching potential of these microbial communities. However, the application of standard molecular biology techniques is especially challenging within these systems due to the nature of the samples and presence of inhibitory compounds. We have successfully employed a range of molecular biology tools, including quantitative real-time PCR, 16S rRNA gene surveys and fluorescent in situ hybridization, to determine the microbial concentrations and assess the profile of the mixed microbial communities involved in bioleaching systems. In addition, we routinely conduct iron and sulphur activity assays to determine the microbial activity of the cultures as well as the effect, if any, on microbial iron oxidation activity due to the presence of soluble inhibitors. The microbial speciation analysis and activity assays provide an additional means for monitoring the performance of bioleaching operations. This information can be applied within commercial bioleaching plants to assess and monitor process upsets and recovery resulting in improved operational management.
Heavy metal contamination in freshwater resources is a global burden. Although intense focus is on remediation strategies there is a need for better detection tools that can be used to monitor the extent of contamination. The aim of this study was to determine levels of heavy metals and link them to dominating bacteria. In this study, water and sediment samples collected from the Crocodile River and six of its tributaries (over wet and dry seasons) were analysed for both chemical and microbial characteristics. The heavy metals were quantified using the inductively coupled plasma mass spectrometer (ICP-MS) and optical emission scanning mass spectrometer (OES-MS). Bacterial diversity was obtained by amplifying the v3 and v4 regions of the 16S RNA gene, followed by separation of the amplicons on the denaturing gradient gel electrophoresis (DGGE). In order to elucidate dominating organisms, dominant DNA bands were recovered from the DGGE gel, sequenced and identified using phylogenetics. Chemical analyses results showed that manganese levels of water samples from the six rivers were higher than 20 ppb, the target water quality range for agriculture. Bacterial analysis revealed that relatives of 4 genera (methylophilus, flavobacterium, limnohabitans and fluviicola) were dominant. The limnohabitans are known to promote the maintenance of species diversity whereas fluviicola species has been found to have a heavy metal tolerance gene. This suggests that the bacterial community could have adapted survival mechanism against heavy metals in the Crocodile river.
Cutinases are enzymes that catalyse the cleavage of the plant polyester, cutin. *Pseudomonas syringae pv. maculicola* (*Psm*) is a phytopathogenic bacteria that infects crucifers. It grows epiphytically on the leaf surface prior to entry into the plant via the stomata and wounds, and multiplies in the intracellular spaces leading to disease development. The genome of *Psm* contains a multitude of sequences annotated as lipases/esterases and a preliminary screening for cutinolytic activity using a polycaprolactone plate assay was positive for this bacterium. As a result, we hypothesised that some of the sequence(s) could represent cutinolytic enzyme(s) that can aid cutin hydrolysis, a trait that could contribute to the epiphytic fitness if maintained during plant leaf colonisation. Therefore, the aim of the study was to perform an *in vitro* characterisation of cutinase production by *Psm* and to identify the enzyme(s) involved. *Psm* was cultured in King’s B medium with cutin (2% w/v) as an inducer as well as uninduced conditions. Cutinase production was monitored using the *p*-nitrophenyl butyrate (*p*-NB) hydrolase and polycaprolactone (PCL) depolymerase assays. Enzyme production was only observed under induced culture conditions and reached a maximum at the stationary phase of growth (40 – 100 h) with both the *p*NB and PCL assay, respectively. A zymogram of the extracellular fraction was performed using 4-methylumbelliferyl butyrate as substrate, and yielded a UV luminescent protein band which was subjected to peptide mass fingerprinting analysis. Similarity searches with the peptide fragments resulted in a hit corresponding to a 30 kD putative lipase from *Psm*. 
Smart hydrogels could facilitate immobilisation of cellulase to allow recovery and decrease enzyme cost in the biofuel industry, as they have a soluble-gel transition. The aim of this study is to design smart hydrogels for immobilisation of cellulase system that can be recovered after hydrolysis of cellulosic biomass. Cellulases from Aspergillus niger FGSC 733, commercial cellulases Aspergillus niger β-glucosidase, were used in immobilisation. Various support matrices prepared were poly-(N-isopropylacrylamide) (p-NIPAAm), poly-(N-isopropylacrylamide-co-Methacrylic acid) (p-NIPAAm-co-MAA) and supermacroporous poly-crosslinked-(Acrylamide-co-N,N'-Methylenebisacrylamide) (p-crosslinked-AA-co-MBA). Hydrolysis of CMC using cellulase-microbeads-p-NIPAAm and cellulase-crosslinked-p-NIPAAm with different percentage gel showed activity trend of 0.05>1>10>5>0.1% and 5>2>10% respectively. HPLC analysis showed that increase of β-glucosidase content in cellulase-crosslinked-p-NIPAAm conjugates increased glucose 12 and 14-fold at 30 and 50 °C respectively in the avicel hydrolysate. Hydrolysis of avicel using cellulase-crosslinked-p-NIPAAm-co-MAA conjugate achieved total of 13.6 g/L of reducing sugars after three re-cycling. Cellulase-crosslinked-p-NIPAAm-co-MAA conjugates was stable than free enzyme at 50 and 60 °C after 24 hour and 120 minute of incubation respectively but lost activities at 65 °C after 120 minute. A total of 21.4 g/L of reducing sugars were released from avicel hydrolysis using cellulase-p-AA-co-MBA conjugate after three re-cycling. In contrast, reducing sugars released in thatch grass hydrolysis using free enzyme were 8 times greater than in cellulase-p-AA-co-MBA conjugate. This shows that cellulase immobilised on smart polymers could be used in hydrolysis of cellulose due to ease of recovery. The sol-gel transition showed to improve recovery of the biocatalyst and therefore contribute in cost reduction of the enzymatic hydrolysis process in the biofuel industry.
The efficiency of cellulases during enzymatic hydrolysis of plant biomass is hampered by their inactivation and non-productive binding to lignocellulosic hydrolysate by-products such as weak acids, furans and lignin residues. The impact may vary from an immediate effect (inhibition) to a gradual decrease in enzyme activity during hydrolysis (deactivation). In this study, the inhibition and deactivation impacts of lignocellulosic hydrolysate were assessed using individual compounds prevalent in hydrolysate and hydrolysate from sugar cane bagasse. Enzymatic hydrolysis was achieved using cellulase monocomponents as well as commercial enzymes. Furthermore, biophysical approaches such as differential scanning fluorimeter (DSF) and circular dichroism (CD) were used to probe the effects of non-productive binding of cellulases onto hydrolysate by-products. The hydrolysate compounds, particularly polymeric phenols and aliphatic acids were observed to selectively inhibit cellulases with a strong effect on cellulbiohydrolase 1 (CBH1) and β-glucosidase 1 (BGL1) but had a moderate inhibitory effect on endoglucanase 2 (EG2). A similar trend was also observed in hydrolysis with the combination of cellulases. However, most monomeric lignin residues had little or no inhibitory effect on hydrolytic enzymes. No significant inactivation was observed suggesting that diminishing enzymatic hydrolysis is largely a function of inhibitor concentration and the enzyme-inhibitor relationship, rather than contact time during the hydrolysis process (i.e. deactivation). The findings in this study suggest that polymeric lignins and aliphatic acids are the strongest inhibitors in hydrolysate.

**Keywords:** Cellulases, Inhibition, Deactivation, Lignocellulosic hydrolysate
Acetyl xylan esterases (AXEs) are Carbohydrate-Active Enzymes (CAZy) that hydrolyse ester bonds to liberate acetic acid in acetylated polymeric xylan and xylooligosaccharides. They form part of a catalogue of enzymes involved in enzymatic saccharification of hemicellulose, a major bottle-neck during bioconversion of lignocellulosic biomass for sustainable biofuel production. While AXEs have been identified from a range of lignocellulose-degrading microorganisms, metagenomic screening methods allow access to novel metabolites/gene products of the ≥90% uncultured microorganisms within any given environmental sample. In this study, a sequence-based approach was employed to screen a Namib Desert soil metagenome for AXE-related ORFs. The Namib Desert is a hot coastal desert possessing unique climatic conditions. In-silico biomining of the Namib soil hypolith metagenomic dataset yielded three (3) putative novel AXE-encoding genes. Sequence homology data showed that these genes designated NaMet1, NaMet2 and NaMet3 possessed 78%, 69% and 51% similarity respectively to other known xylan deacetylases. The novel ORFs shared the GXSXG and GDS(L) conserved motifs characteristic of the CE7 (NaMet1&2) and CE3 (NaMet3) CAZy families, respectively. All three possessed the Ser-His-Asp(Glu) catalytic triad. Active site residues synonymous with SGNH hydrolases were located at Ser49, Gly106, Asn163 and His225 of NaMet3. These genes have been synthesised and are currently undergoing expression and functional characterisation. These results suggest that novel functional AXEs may be discovered via sequence-based metagenomics.
Bisphenol-A (BPA) is a high-volume chemical mostly used in the production of plastics. It has been identified as a causative agent of adverse health effects in both humans and animals. In this study, the biodegradation of BPA using two fungal laccases \( \text{Trametes versicolor} \) (TvL) and \( \text{Trametes pubescens} \) (TpL), a bacterial laccase \([\text{Streptomyces coelicolor (SLAC)}]\) and a mutated variant of the small laccase (SLAC-VN) was investigated. BPA biodegradation was determined using free laccases, encapsulated (sodium alginate) laccases, as well as cross-linked enzyme aggregates (CLEAs). The effect of pH and temperature conditions was determined for all enzyme preparations, as well as the stability and reusability of the immobilised laccases.

The highest BPA removal was exhibited by free TpL (100% removal) and free SLAC-VN (96% removal). Overall, the BPA removal level was reduced for all encapsulated enzymes when compared to the free laccases. Sodium alginate encapsulation also increased the optimal temperature for BPA removal for all encapsulated laccases. The formation of CLEAs had the most significant effect on the BPA removal by the laccases: the formation of CLEAs enhanced the BPA removal capability of SLAC with a 60% removal compared to 25% when encapsulated. LC-MS analyses of putative BPA metabolites showed that BPA was mainly oligomerised into dimers, trimers and tetramers. Oestrogenic screening showed that the oestrogenic activity of BPA was completely reduced for free TvL and TpL, and a 98% reduction was observed for free SLAC-VN. Here we have shown that a bacterial laccase can biodegrade BPA to the same extent as fungal laccases.
S2.07 - AN EVALUATION OF THE EFFECTS ON THE NON-CATALYTIC GALACTOMANNAN BINDING ABILITY OF β-MANNOSIDASES ON THEIR ACTIVITY AND SYNERGISM WITH A MANNANASE DURING GALACTOMANNAN HYDROLYSIS

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The hydrolysis of galactomannan to mannose requires the synergistic action of β-mannanases and β-mannosidases. This study assessed the substrate specificities and galactomannan binding abilities of two β-mannosidases of GH families 2 (BtMan2A) and 5 (CmMan5A). Thereafter, their synergistic associations with a β-mannanase during galactomannan hydrolysis were evaluated to elucidate how differences in their features played a role in their synergistic interactions. CmMan5A displayed higher activity on galactose-containing oligomers and galactomannan polysaccharides compared to BtMan2A, which was more active on pNPM. During their interaction with galactomannan, it was found that the β-mannosidases bound reversibly and irreversibly to the substrate, with BtMan2A exhibiting higher affinity for the biomass. A binary enzyme combination ratio between a mannosidase and mannanase required to maximize the release of both reducing sugar and mannose residues from galactomannan was determined. Results from the binary mannanolytic enzyme homeosynergy studies showed that more reducing sugars were released with the enzyme combinations containing CmMan5A (1.2 fold) compared to those combinations with BtMan2A (≈1.0 fold). From the findings obtained in this study, it was observed that during galactomannan degradation, the synergistic association between BtMan2A and ManA was affected by the low specific activity of BtMan2A towards galactose-containing oligomers, as well as its non-catalytic galactomannan binding ability.
World-wide, tuberculosis ranks as the second leading cause of mortality from an infectious disease. Diagnostic delays pose the most significant problem and accurate point of care diagnosis is unavailable. The GeneXpert platform does not comply with all point of care test criteria and may not be sustainable for low income countries. Mycobacterial pili (MTP) have been characterised extensively and found to be unique to *M. tuberculosis* complex (MTBC) pathogens.

In this study Real Time PCR was used to assess *mtp* gene expression in MTBC pathogens and non-tuberculous mycobacteria (NTM) in bacilli grown under hypoxic conditions and in a hypoxic biofilm of the Beijing, F11, F28 and F15/LAM4/KZN strains. All MTBC strains expressed the *mtp* gene *in vitro* and the NTMs had no detectable gene expression. This validates MTP protein as potential diagnostic biomarker. Beijing, F11 and F28 strains had significantly downregulated *mtp* gene expression in the anaerobic planktonic and biofilm cultures, whereas KZN strain upregulated *mtp* gene expression under these conditions. MTB have been shown to have a lower infectivity profile under anaerobic conditions. This data thus indicates the different rates at which the more virulent strains can enter dormancy compared to the less virulent counterparts. This highlights the importance of characterising potential biomarkers in in different strains of *M. tuberculosis* in aerobic and anaerobic cultures. The potential of MTP as a diagnostic marker needs to be further studied to include screening for MTP in latently infected and active tuberculosis patients.
Cassava (Manihot esculenta Crantz) is the world’s fifth most important staple food crop. Cassavas main value comes from its roots, which have a high carbohydrate content. Cassava displays high tolerance to abiotic stresses and adverse environments, making it an important crop for subsistence farmers in resource poor environments. Cassava mosaic disease (CMD), caused by a number of begomoviruses belonging to the Geminiviridae family, is the most important disease of cassava where losses due to CMD can be up to 100%. The most viable method of controlling CMD is through the production of resistant crops using transgenic technologies. Post-transcriptional gene silencing (PTGS), a natural defense mechanism found in plants, can be enhanced using transgenic technologies to increase the plants ability to resist infection by specific viruses. This technology may increase cassava ability to resist infection by the viruses associated with CMD.

The aim of this project is to develop CMD virus resistant cassava cultivar. In this study, a triple stacked construct which targets the AC1/AC4 region of three main geminiviruses associated with CMD in Southern Africa (SACMV, ACMV and EACMV) has been constructed and has been transformed into cassava cv. 60444. The ability of the construct to induce resistance is currently being evaluated against each of the viruses individually as well as all three viruses together, and results will be presented.
Molecular biology based methods such as real-time polymerase chain reactions (qPCR) are a powerful alternative to standard methods for the routine monitoring of environmental samples. These assays are highly specific and can ensure rapid detection and quantification of intestinal parasites such as *Cryptosporidium parvum* and *Ascaris lumbricoides*. The primary aim of this study was to optimise real-time PCR protocols coupled with high resolution melt (HRM) curve analyses for the detection and quantification of *C. parvum* oocysts in faecal and drinking water and *A. lumbricoides* ova in sludge. The DNA extraction efficiency of three different kits coupled with beads and freeze-boil cycles were compared. In addition, real-time PCR reagents and conditions were compared and optimised. Standard curves were also set up using gBlocks™ Gene Fragments, with the detection limits calculated. The QIAamp Fast DNA Stool Minikit yielded the best DNA extraction and subsequent qPCR results for the *C. parvum* oocysts. The same kit coupled with beads and freeze-boil cycles also proved to be the most effective for DNA extraction and qPCR analysis of *A. lumbricoides* ova. Melting profiles obtained from faecal and sludge samples were characterized by peaks of 79.48 ± 0.24 °C and 78.10 ± 0.26 °C for *C. parvum* and *A. lumbricoides*, respectively. This study successfully optimised a qPCR procedure coupled with HRM analyses for the detection and quantification of *C. parvum* oocysts from faecal and spiked drinking water samples. A novel qPCR procedure coupled with HRM analyses was also developed for the detection and quantification of *A. lumbricoides* ova from sludge samples.
Disinfectants are commonly used to control the spread of bacteria. Quaternary ammonium compound-based (QAC) disinfectants are therefore commonly used in the clinical, veterinary and food environments. Since the problems occurring with antibiotic resistance, several restrictions have been placed on the use of antibiotics in animal production. Therefore alternatives must be in place to help control the spread of bacteria. A good disinfection programme is therefore important in the veterinary industry. However, low-level resistance to QACs have been observed, especially in *Staphylococcus aureus*, and some of the associated genes have already been identified. In this study, bacteria isolated from poultry pens were screened using PCR for four of the *qac* resistance genes (*smr, qacJ, qacH* and *qacG*). Minimum inhibitory concentrations of three QAC-based disinfectants have been determined for the field isolates in order to relate the presence of the resistance genes to an increase in tolerance to the QACs. The results obtained from this study indicates that bacteria can contain multiple *qac* resistance genes, and the presence of the genes did not necessarily result in a higher tolerance to QACs. Since knowledge on the substrate specificity of these genes are lacking, and presence of numerous *qac* resistance genes in one isolate may indicate the ability to adapt rapidly to environments where QACs and or antibiotics are frequently used.
Two episomal begomovirus-associated sequences, named Sequences Enhancing Geminivirus Symptoms (SEGS1 and SEGS2), were identified in field cassava affected by the devastating cassava mosaic disease (CMD). The sequences reportedly exacerbated CMD symptoms in the tolerant cassava landrace TME3, and the model plants *Arabidopsis thaliana* and *Nicotiana benthamiana*, when biolistically co-inoculated with *African cassava mosaic virus*-Cameroon (ACMV-CM) or *East African cassava mosaic virus*-UG2 (EACMV-UG2). This is highly relevant as cassava is an important food security crop in Sub-Saharan Africa. Following the identification of small SEGS fragments in the cassava EST database, the intention of this study was to investigate a possible role for these sequences in CMD since the endogenous iSEGS1 and iSEGS2 are in close proximity or overlapping with cassava genes, suggesting a possible role in regulation of specific biological processes. We confirm the expression of iSEGS *in planta* using real time RT-PCR. A bioinformatics study revealed that the sequence features of genome-integrated SEGS (iSEGS) are unique but resemble non-autonomous transposable elements (TEs) such as MITEs and helitrons. It is interesting to note that helitrons are circular and can replicate via rolling circle replication. Furthermore, many iSEGS-associated genes, some involved in virus-host interactions, are differentially expressed during infection by *South African cassava mosaic virus* (SACMV) of susceptible (T200) and tolerant (TME3) cassava landraces. Differentially abundant SEGS-derived small RNA populations were present in mock-inoculated compared with SACMV-infected T200 and TME3 leaves. We conclude that, given the known role of TEs and associated genes in gene regulation and plant immune responses, our observations are consistent with a role of these DNA elements in the host’s regulatory response to geminiviruses.
The yeast *Kluyveromyces marxianus* is emerging as a potential host for metabolic engineering and recombinant protein production, having a number of advantages over *Saccharomyces cerevisiae*. To date, very little is known about its genetic and metabolic responses to various nutritional environments. A new draft genome sequence and annotation of a South African isolate is reported here as the blueprint for integrative systems biology. In addition, our RNA-seq transcriptomic data indicates a large number of genes differentially regulated in response to different sugars in the cultivation medium. Gene set enrichment using ontologies effectively reveals differential regulation of sugar transporters, cofactor regenerating pathways and, unexpectedly, peroxisomes and lipid metabolism. Subsequently, a biochemical networks approach to exploring omics data is used to extract likely cause-and-effect relationships in the genetic programme of this yeast, identifying transcription factors that likely play a role in the sugar response. New software for integrative systems biology is presented.
Starch is one of the most commonly used feedstocks for ethanol production but current starch-to-ethanol processes require an energy-intensive liquefaction step, as well as considerable amounts of exogenous enzymes. Amylases are widely used in a number of different industrial processes for the hydrolysis of starch and account for 25-33% of the international enzyme market; however, only about 10% of amylolytic enzymes are able to hydrolyse raw or unmodified starch. The most economical way to produce ethanol from raw starch is by using a single organism or microbial consortium that is able to degrade the starchy biomass and ferment the resulting sugars; this approach is called consolidated bioprocessing (CBP). Despite its efficiency as an ethanol producer, *Saccharomyces cerevisiae* cannot produce ethanol from raw starch directly, because it lacks the ability to degrade starch into glucose. Therefore, in order to construct a CBP organism, recombinant strains are required that express amylolytic enzymes with a combination of α-amylases and glucoamylases as a minimum requirement for complete hydrolysis. The use of an amylolytic fermentative organism will not only produce ethanol, but its inclusion in an SSF process will decrease the reliance on commercial enzymes. This study focuses on the direct ethanol production from starchy biomass using recombinant enzymes for the hydrolysis of raw starch. We examine the use of recombinant cellulases and engineered amylase-secreting strains for the hydrolysis and saccharification of wheat bran, as well as demonstrate the efficient conversion of grain sorghum and triticale using a CBP yeast.
Biohydrogen has been identified as a promising alternative to the use of non-renewable fossil reserves, owing to its sustainability and non-polluting nature. pH is considered as a key parameter in fermentative biohydrogen production processes, due to its effect on the hydrogenase activity, metabolic activity as well as substrate hydrolysis. The present study assesses the influence of regulating pH on dark fermentative biohydrogen production. Four experimental hydrogen production schemes were evaluated. Two were implemented using suspended cells under regulated pH growth conditions (Sus_R) and suspended and non-regulated pH (Sus_N). The two others regimes consisted of alginate immobilized cells under pH regulated growth conditions (Imm_R) and immobilized and non-pH regulated conditions (Imm_N). All experiments were carried out at 37.5°C with glucose as sole source of carbon. Sus_R showed a lag time of 5 hours and a peak hydrogen fraction of 36% and a glucose degradation of 37%, compared to Sus_N which showed a peak hydrogen fraction of 44% and complete glucose degradation. Both suspended culture systems showed a higher peak biohydrogen fraction compared to the immobilized cell system. Imm_R experiments showed a lag phase of 8 hours, a peak biohydrogen fraction of 35%, while Imm_N showed a lag phase of 5 hours, a peak biohydrogen fraction of 22%. 100% glucose degradation was observed in both pH regulated and non-regulated processes. This study showed that biohydrogen production in batch mode with suspended cells in a non-regulated pH environment results in a partial degradation of substrate, with lower yield. This scheme has been the culture mode of choice for most reported studies in biohydrogen research. The relatively lower slope in pH trend of the non-regulated pH experiment with immobilized cells (Imm_N) compared to Sus_N revealed that that immobilized systems have a better buffering capacity compared to suspended systems, which allows for the extended production of biohydrogen even under non-regulated pH conditions. However, alginate immobilized cultures in flask systems showed some drawbacks associated to high rate of gas production that leads to increased buoyancy of the immobilization beads. This ultimately impedes the release of gas out of the flask.
Filamentous fungi represent an interesting source of pigments which could address the consumer-driven move toward natural pigment alternatives in food, health and nutraceutical products. At present, the majority of natural pigments are obtained from sources such as plants and insects, and are therefore affected by limitations such as natural variation and seasonal availability. Filamentous fungi produce a wide variety of pigments under conditions which can be easily controlled, with minimal dependence on weather and seasonal raw materials. A factor which may limit the application of these pigments, however, is the production of toxic secondary metabolites by some fungal species. A literature review of known fungal pigment producers was performed in order to allow selection of organisms for production studies. This review considered factors such as pigment type and colour, fungal growth requirements, and the production of toxic metabolites. Two fungal species were selected for investigation, *Epicoccum nigrum* and *Penicillium purpurogenum*. These produce intracellular and extracellular pigments of a variety of colours, without the co-production of mycotoxins. Initial cultivation studies confirmed effective pigment production by these organisms. The impact of changing medium composition and cultivation conditions was determined, with the aim of identifying conditions which are beneficial for pigment production. This was assessed using a variety of cultivation methods, including agar plates, shake flasks and bench-top bioreactors, and production across these growth scales was compared.
ENGINEERING BACTERIOPHAGE RESISTANCE IN GEOBACILLUS THERMOGLUCOSIDASIUS TOWARDS A ROBUST PLATFORM FOR BIOFUELS PRODUCTION

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Geobacillus thermoglucosidasius is a promising “platform” organism to use in the production of a range of useful metabolites with demonstrated ability to produce ethanol, isobutanol and polylactic acid for bio-degradable plastics. It is well known that commercial bacterial fermentations are prone to bacteriophage attack, and as such any strains used either have to be engineered, or selected for resistance against the phage. Although several Geobacillus spp phages have been described (GVE1, GVE2, GBSV1, GBK2, DE6 and φOH2), sequenced, and one in particular GVE2, well studied, none have been found that infect G. thermoglucosidasius. Here we describe a novel bacteriophage that infects G. thermoglucosidasius (GVE3) and we attempt to develop strains resistant against the phage. One solution was the over-expression of the phage “immunity” protein encoded by GVE3. Another, was to identify spontaneous GVE3 resistant mutants. The high lysogen background had to be reduced through over expression of a suspected cl-like regulator protein to ensure lytic conversion of all infected cells. Next generation sequencing of a mutant showed an amber mutation in the polysaccharide pyruvyl transferase (csaB). A double crossover knockout of csaB confirmed the phenotype and resulted in phage resistance. Several other target proteins were also expressed to determine if they could effect phage resistance including a potential anti-holin, HTH-like regulator as well as inactivation of yueB, however none of these resulted in a phage resistance phenotype.
S4.06 - ENZYMATIC SACCHARIFICATION OF PRE-TREATED BANANA PSEUDOSTEM AND FERMENTATION OF THE HYDROLYSATE TO ETHANOL

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Banana pseudo-stem (BPS) is a by-product that remains after the harvest of banana fruit. BPS is a promising feedstock for ethanol production due to its high cellulose and hemicellulose content. The aim of this study was to hydrolyse banana pseudostem with the cellulase produced by locally isolated fungus *Trichoderma longibrachiatum* LMLUL 14 – 1 and commercial cellulase. BPS was subjected to three different pre-treatments namely liquid hot water (LHW), 5% H$_2$SO$_4$ and 3% NaOH at 121 °C for 60 min. Crude enzyme from *T. longibrachiatum* LMLUL 14 – 1 with cellulolytic activity (FPase 67 FPU/g ds) and commercial cellulase with cellulolytic activity (FPase 57 U/ml) were added to 10 g of each pre-treated BPS in 10 ml of 0.05 M sodium citrate buffer, pH 5 at 50 °C for hydrolysis. Hydrolysis of NaOH pre-treated BPS resulted in high glucose released for both crude and commercial enzyme (21 g/L and 35 g/L, respectively) when compared to other pre-treatment strategies. Fermentation of the liquid fraction (pre-hydrolysate) from the above treatments resulted in 15.7 g/L of ethanol produced from H$_2$SO$_4$ pre-hydrolysate, 1.2 g/L of ethanol from LHW pre-hydrolysate and no ethanol produced from NaOH pre-hydrolysate. This study reveals the potential of BPS to produce sugar (glucose and xylose) and its application in the bioethanol industry.
Cyanate and its by-products have been extensively used as herbicides as well as precursors in the synthesis of polymers and is released into the environment. Furthermore, cyanate is also released by spontaneous cyanide photooxidation, otherwise, by the oxidative treatment of cyanide-containing wastes. Active form of cyanate i.e., isocyanic acids, act as a potential toxin as it carbamoylates amino acids, proteins, and other molecules which cause changes in their structure, charge and function. Cyanate hydratase transforms cyanate to CO\textsubscript{2} and NH\textsubscript{3} in a bicarbonate-dependent reaction. Fungi are tremendously useful in booming out biotransformation processes which become progressively important in biocatalysis and biorefinery applications. Cyanate hydratase has potential applications in bioremediation to reduce environmental pollution especially by cyanate. However, the enzyme production by wild microbial strains hardly ever meet cost-effective production on an industrial scale. The present investigation therefore focuses on the cloning, expression and characterization of cyanate hydratase from a thermophilic fungus, *Thermomyces lanuginosus*. The Cyn gene, encoding cyanate hydratase of *T. lanuginosus* was amplified from the cDNA and has been cloned into pET21b vector and the resulting plasmid was transformed into *Escherichia coli* BL21 (DE3). Among the *E. coli* clones screened, one positive clone was selected for further investigation and observed a 20-fold higher cyanate hydratase titre than the native host *T. lanuginosus*. An expression band of 18 kDa was observed on SDS-PAGE which is similar to the theoretical value. Current research focuses on improving the enzyme production and characterization of the recombinant cyanate hydratase.
Microalgae have great potential as a renewable feedstock for biodiesel production. The process still has various limitations, one of which is the utilisation of large amounts of water and chemical media. The use of wastewater reduces the requirement for freshwater supplies. This study evaluated the growth of Chlorella sorokiniana on pre- and post-chlorinated domestic wastewater effluent to assess its potential as a medium for microalgal growth and lipid production. The residual nitrogen in post-chlorinated effluent supported microalgal growth for 18 d. Supplementation of post-chlorinated effluent using a cheap nitrogen source was required for optimal biomass and lipid production. Urea, potassium nitrate, sodium nitrate and ammonium nitrate were evaluated in terms of microalgal biomass and lipid production. Urea showed the highest biomass yield (0.216 g L\(^{-1}\)). Urea concentrations (0–10 g L\(^{-1}\)) were assessed for its effect on growth and microalgal physiology using pulse amplitude modulated fluorometry. A concentration of 1.5 g L\(^{-1}\) urea produced 0.218 g L\(^{-1}\) biomass and 61.52% lipid by relative fluorescence in 24 d. Physiological stress was evident by the decrease in relative Electron Transport Rate from 10.45 to 6.77 and quantum efficiency of photosystem II charge separation from 0.665 to 0.131. Gas chromatography analysis revealed that C16:0, C18:0, C18:1, C18:2 and C18:3 were the major fatty acids produced by C. sorokiniana. Wastewater effluent is an important resource for economical and sustainable microalgal biomass/lipid production. The results indicate that supplemented wastewater effluent was an acceptable alternative to conventional media. Using a relatively cheap nitrogen source like urea can certainly improve the techno-economics of large scale biodiesel production.
The aim of the study was to compare the efficiency of a locally produced closed-coupled solar pasteurization system to an internationally designed system for the treatment of harvested rainwater. To achieve this aim, rainwater samples were collected before and after treatment at the temperature range of 70 to 85°C. These samples were analysed by enumerating indicator bacteria, performing ethidium monoazide-qPCR (EMA-qPCR) to monitor the viability of *Legionella* spp., and monitoring ATP levels using the BacTiter Glo™ Assay. Preliminary results suggest that both solar pasteurization systems are effective in reducing the level of indicator organisms in the tank water to within drinking water standards at temperatures above 72°C. In addition, a 99.6% and a 97.5% reduction in ATP levels was obtained after pasteurization for the locally designed and international model systems, respectively. EMA-qPCR analysis then indicated that the locally designed system reduced genomic copy numbers of intact *Legionella* cells by 79% (72°C), 84% (77°C), 93% (81°C) and 99% (84°C), while the international system indicated a mean decrease of ~99% for all temperatures tested. Chemical parameters were also monitored to determine whether the chemical quality of the water was altered during treatment and while the concentrations of lead, nickel, iron and aluminium were detected at levels exceeding recommended guidelines in tank water pasteurized using the international model, only lead concentrations exceeded the recommended guidelines for the rainwater pasteurized using the locally designed system. Pilot-scale studies are thus currently being conducted in Enkanini informal settlement to determine the treatment efficiency and sustainability of utilizing solar pasteurization for the treatment of rainwater.
Carbon sequestration by microalgae is the most efficient form of remediating the greenhouse gas, CO₂. The effect of different levels of carbon sources on microalgal growth was investigated in order to identify a high carbon tolerance environmental strain for industrial carbon sequestration. Five algal isolates and a reference strain, *Coccolithus pelagicus* (coded I-0) were subjected to concentrations of between 0.03 and 15% CO₂ and 0.05 and 2 g·CO₂·L⁻¹ NaHCO₃ over 21 days. Of the five, strain I-3 recorded the highest $\mu_{\text{max}}$ of 0.418 d⁻¹ and 0.392 d⁻¹ respectively at high (10 and 15%) levels of CO₂. The maximum biomass obtained ($B_{\text{max}}$) of I-3 significantly increased from 214 to 828 mg·L⁻¹ when CO₂ concentration was raised from 0.03% to 15%. At each NaHCO₃ concentration, either the reference strain (I-0) or the indigenous isolate I-3, yielded the highest biomass. Additionally, the $\mu_{\text{max}}$ of I-3 rose with an increase in NaHCO₃ concentration and reached 0.408 d⁻¹ at 2 g·CO₂·L⁻¹. The $B_{\text{max}}$ of I-3 increased from 153 mg·L⁻¹ (at 0.05 g·L⁻¹) to 774 mg·L⁻¹ at (2 g·L⁻¹). Isolate I-3 showed similar biomass production values to the reference strain (I-0) ($p > 0.05$). The $B_{\text{max}}$ of strain I-3 was higher than the other strains at all CO₂ concentrations. Relative electron transport rate and maximum quantum yield were used to assess the physiological impact of elevated CO₂ and NaHCO₃ concentrations. The decline in maximum quantum efficiency for strain I-3 and the reference strain (I-0), at varying NaHCO₃ concentrations was similar ($p > 0.05$). Isolate I-3 also displayed the highest electron transport rate (rETR) confirming its tolerance to higher concentrations of CO₂ and NaHCO₃. Based on partial 28s rRNA gene sequencing, the successfully isolated high-carbon sequestering strain I-3 was found to be homologous to the ribosomal genes of *Chlorella* sp.
All spheres of the ecosystem, including the soil, have been affected by pollution with different xenobiotics, which include polycyclic aromatic hydrocarbons (PAHs). PAHs are a problem due to their persistence in the soil. Although many PAHs have highly toxic, carcinogenic and mutagenic properties a variety of bacteria can degrade them. Degradation of PAH by bacteria is due to adaptation mechanisms that enables them to survive in polluted soil. The aim of this study was to isolate PAH degrading bacteria from soil after 10 weeks of artificial PAH contamination. Soil with no history of PAH contamination was excavated to a depth of not more than 25 cm in the garden of the Agricultural Research Council - VOPI. It was subjected to two treatments, A and B. Treatment A was a three-level contamination of the soil with used engine oil while treatment B was a three-level biostimulation of the soil with vermicompost. A total of 68 bacterial strains were isolated from the different treatments after selective enrichment on Bushnell Haas broth and agar with three PAHs as a source of carbon. Forty isolates were identified on the basis of their 16s rRNA gene. The following genera were identified: Acinetobacter, Arthrobacter, Bacillus, Microbacterium, Ochrobactrum, Pseudomonas and Strenotrophomonas. The bacterial isolates demonstrated different PAH degradation abilities and tolerance.
Soil pollution with persistent organic pollutants, especially polycyclic aromatic hydrocarbon (PAHs) is a global menace and its remediation is imperative primarily for health reasons, as PAHs tend to biomagnify through the food chain. Bioremediation is favoured over other forms of remediation and is particularly promising for being economical and environmentally safe. Bacteria isolates with diverse capabilities of bioremediation and enhancing soil fertility are in high demand where they are envisaged to be of great benefit to sustainable agricultural production. Polycyclic aromatic hydrocarbon-degrading bacteria were isolated from enriched cultures of engine-oil polluted soil. The isolates were identified by partial 16S rRNA sequence analyses as members of the phyla Firmicutes, Actinobacteria and Proteobacteria. They were investigated for their ability to solubilise insoluble form of phosphate, fix atmospheric nitrogen and produce indoleacetic acid (IAA) as a way to ascertain their potential capabilities of contributing to soil fertility. The Proteobacteria displayed the greatest potential for soil fertility enhancement. The genome of one of the Proteobacteria isolates was sequenced and annotated and found to possess several dioxygenases, reductases, ferredoxin, dehydrogenases and Rieske proteins all known to play an active role in biodegradation processes. Other genes, such as phosphatases known to play an active role in phosphates solubilisation and 1-aminocyclopropane-1-carboxylate deaminase, known to promote root elongation, were also identified on the genome. These findings demonstrate a clear potential of the isolates to participate in restorative bioremediation of polluted soils which is of immense benefit in the agroforestry and mining sectors.
Aquaculture, also known as aquafarming, is the farming of aquatic organisms such as fish, crustaceans, molluscs and aquatic plants. Aquaculture production of fish and molluscs is worth billions of rands per year. A significant limitation to the industry is loss of stock through bacterial disease. Traditional methods to combat disease with antibiotics have been questioned and alternatives have been sought. The field of probiotics as well as the screening methods used to acquire probiotic strains for the alternative management of disease needs to be investigated. Thus, the aim of this research was to identify probiotics for Tilapia by screening bacteria which produce inhibitory compounds, has the ability to survive the pH of gastric acid, has digestive enzyme activity can compete for adhesion sites and can enhance immune response.

Samples were obtained from Tilapia ponds around Durban. The swabs were plated and Bacillus selected, their identity established by performing the Gram stain, endospore stain and catalase test. The resultant 44 strains isolated were evaluated for anti-pathogenic activity against Aeromonas hydrophilia. Results show that all 44 strains possess inhibited and thus they had anti-pathogenic activity. pH tolerance was measured using pH values ranging from 3 to 11. All strains grew within the tested pH range. Presence of digestive enzymes: cellulase; protease; lipase and amylase was measured using enzyme assays. Degradation activity corresponding with these enzymes was noted in all assays. Growth kinetics was measured spectrophotometrically over 12 hours. Most strains showed favourable growth kinetics. The isolated strains exhibited activity to varying degrees, thus a weighting matrix was formulated, scoring the isolates against a theoretical positive control as well as a fish feed probiotic control. Bacillus strains TS 098, TW 099, TS 101 TW 105, TS 106, TF 107 and CT 108 were selected according to this matrix.

The results of this study indicate the potential modes of action of a probiotic feed additive. Eight of the isolates exhibited superior competitive exclusion of A. hydrophilia, ability to tolerate a range of pH, including very low pH, and favourable enzyme activity. The eight selected strains were further evaluated for quantitative enzyme activity as well as ability to colonise the gut epithelia of the host using Caco2 cell model. Immunomodulatory activity of strains were be measured using flow cytometry.
Endophytic bacteria colonize the internal tissues of their host plants and display a range of different symbiotic relationships. Endophytes associated with medicinal plants have gained attention in pharmaceutical field as they produce similar compounds as their host, which possess a variety of bioactivities. The diversity and bioactivity of endophytic bacteria associated with indigenous South African medicinal plant *Kigelia africana* was, therefore, investigated. Leaf samples were collected, surface-sterilized, processed and incubated on selective and non-selective media. Isolates were subjected to primary antimicrobial screening, ethyl acetate extractions and secondary (agar-well diffusion assay) assays. A quorum-sensing sandwich assay was used to screen for quorum quenching isolates using three biosensor systems. Twenty bacterial extracts were used to quantify the level of quorum quenching using pyocyanin and violacein inhibition assays. Two hundred and fifty-seven bacterial cultures (91% Gram-positive) with varying colony and cellular morphologies were isolated. Primary antimicrobial screening identified susceptibility in the following order: *Escherichia coli* (37%) > *Klebsiella pneumoniae = Staphylococcus aureus* (30%) > *Enterococcus faecalis* (2.7%) > *Pseudomonas aeruginosa* (1.4%). Following ethyl acetate extractions of 20 selected isolates, significant increases in antimicrobial activity were observed. Using the sandwich assay, 69% isolates degraded short chain acyl homoserine lactones (AHLs), 52% intermediate length AHL chain degradation and 52% degraded long chain AHLs. Decreased pyocyanin and/or violacein production, without significant effects on growth, were observed for selected extracts. Bacterial endophytes from *K. africana* could potentially be a source of new, effective anti-virulence drugs for antimicrobial therapy.
Crustacean waste produced from industrial processing of seafood, such as shrimp, crab, prawn and lobster can produce several bioactive molecules. The present study reports extraction of bioactive molecules such as chitin, chitosan (CH), glucosamine (GN) and chitooligomers (COS) from shrimp waste using chemical methods. Chitin was recovered from processing waste with a yield of 30% (w/w) which was further used to obtain 60 g of 72% deacetylated chitosan per 100 g of chitin. Chitin and CH can be hydrolysed into glucosamine and their respective oligomers in 2 h with 32% HCl at 60°C and 80°C, respectively. The oligomer mixed fractions were desalted by activated charcoal extraction and components of each fraction were analysed by TLC and HPLC. COS and N-acetyl chitooligomers (NACOS) with degree of polymerization (DP) ranging from 2-6 were obtained. Chitosan (1%) was more effective against Gram-positive bacteria, while the oligomers were ineffective.

Additionally, we produced chitosan films using glycerol, polyethylene glycol 200 (PEG-200) and polyethylene glycol 600 (PEG-600) as plasticizers. Films were characterized by FTIR, X-ray diffraction, TGA, DMA and SEM. The tensile, barrier, and sorption properties of the films were also evaluated. The CH film yielded mechanically resistant films without the use of a plasticizer. CH film and CH film with glycerol blend were translucent in appearance and opaque in blend with PEG-200 and PEG-600. These results indicate the feasibility of an integrated process for isolating highly bioactive molecules, such as oligosaccharides, with a broad spectrum of applications from shrimp processing waste.
Bioengineered probiotic *Lactobacillus casei* expressing listeria adhesion protein (LAP) have been shown to reduce *L. monocytogenes* adhesion to, invasion into and translocation across Caco-2 cells. However, whether this bioengineered strain will affect pathogenicity of *L. monocytogenes* and other Gram negative pathogens in simulated intestinal conditions under anaerobic conditions remain unknown. This study aimed to investigate the effect of LAP- expressing *L. casei* on *L. monocytogenes* and *S. Typhimurium* in simulated intestinal fluid under anaerobic conditions. The mammalian cells were grown to confluency and then pretreated with probiotics before being exposed to pathogens, both suspended in simulated intestinal fluid, followed by incubation under anaerobic conditions. Adhesion and invasion of pathogens were analysed in vitro using Caco-2 and HCT-8 cell models, and translocation of the pathogens was determined using transwell model pre-seeded with Caco-2 cells. Bioengineered *L. casei* inhibited adhesion to, invasion into and translocation across intestinal cells under the test conditions, and reduced its cytotoxicity onto the epithelial cells but it did not reduce the adhesion of *S. Typhimurium*. Tight junction integrity analysis using dextran fluorescein iso thiocyanate (Dextran<sup>FITC</sup>) indicated that lower percentage of Dextran<sup>FITC</sup> was recovered from basolateral chamber for Caco-2 cells pre-treated with recombinant *L. casei* prior to *L. monocytogenes* exposure. Furthermore, transepithelial electrical resistance (TEER) analysis revealed lower TEER reduction for cells pre-treated with bioengineered *L. casei*. These results showed specificity of LAP for interaction of *L. monocytogenes* without offering enhanced cross protection against *S. typhimurium*. 
S6.03 - COCKTAILS OF PROBIOTICS PRE-ADAPTED TO MULTIPLE STRESS FACTORS ARE MORE ROBUST UNDER SIMULATED GASTROINTESTINAL CONDITIONS THAN THEIR PARENTAL COUNTERPARTS AND EXHIBIT ENHANCED ANTAGONISTIC CAPABILITIES AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

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The success of the probiotics depends on their ability to withstand the stressful conditions; hence development of robust cultures is critical to the probiotic industry. The combination of probiotic cultures has proven to be more effective than the use of single cultures. We investigated the effect of the pre-adaptation of the probiotics to multiple stresses and the effect of their singular as well as their synergistic antagonistic effect against selected enteric pathogens. Probiotic cultures were inoculated into MRS broth (pH 2) and incubated for 2 h at 37°C. Survivors were subcultured into 2% bile acid for 1 h at 37°C. Survivors were finally inoculated in fresh MRS broth and incubated at 55°C for 2 h. The cells surviving were then used as stress adapted cultures. The adapted and non-adapted cultures were exposed to simulated gastrointestinal conditions. The combination cultures were tested for their antipathogenic effects on E. coli and S. aureus. Acid and bile tolerances of most of the stress-adapted cells were higher than of the non-adapted cells. Viable counts of all the stress-adapted lactobacilli and B. longum LMG 13197 were higher after exposure to simulated gastrointestinal fluids. A cocktail containing L. plantarum + B. longum Bb46 + B. longum LMG 13197 best inhibited S. aureus, while E. coli was best inhibited by a combination containing L. acidophilus La14 150B + B. longum Bb46 + B. bifidum LMG 11041. Multi-stress pre-adaptation enhances viability of probiotics; and mixture of multi stress-adapted cells exhibits enhanced synergistic effects against foodborne pathogens.
Free-living amoebae (FLA) isolated from aquatic environments, such as *Naegleria fowleri*, *Balamuthia mandrillaris* and *Acanthamoeba* species, have been implicated in central nervous system, eye and skin infections. They also allow the survival, growth and transmission of bacterial species belonging for example to the genus *Legionella*, *Mycobacteria* and *Vibrio* in water systems. The purpose of this study was to investigate the occurrence of potentially pathogenic FLA and their associated bacteria in three selected hospital water networks in Johannesburg. A total of 275 water (143) and swab (132) samples were collected from the inlets, theatres, intensive care units, endoscopy units, renal units, neonatal ward, milk room, diarrhoea ward and sterilization units. The samples were filtered and analyzed using an amoebal enrichment technique. FLA were isolated in 154 (56.0%) (71 water and 83 biofilm). Using microscopy, PCR and 18S rRNA sequencing, *Acanthamoeba* spp. (T1, T3 and T20 genotypes), *Vermamoeba vermiformis* and *Naegleria* spp. were identified. The most represented bacterial species detected in the three hospitals were *Aeromonas salmonicida*, *Sphingomonas paucimobilis*, *Delftia acidovorans*, *Comamonas testosterone*, *Serretia marcescens* and *Stenotrophomonas maltophilia*. All these bacterial species have been linked with opportunistic human infections. Therefore any exposure to immune-compromised patients to FLA and associated bacteria from the water distribution systems poses a potential health risk. To our knowledge, this is the first study in South Africa and will therefore provide baseline information to infection control personnel on a potential source of infection in a hospital environment.
Introduction: Pneumonia is the most common cause of mortality in children under the age of five years, with the highest burden in African children. Few studies have profiled the nasopharyngeal (NP) microbiome in children with pneumonia in comparison to healthy controls.

Objective: We aim to investigate the role of infant NP bacterial profiles in pneumonia pathogenesis by comparing bacterial profiles of NP specimens collected longitudinally from infants (at 2-weekly intervals) preceding the onset of pneumonia, and cross-sectionally at the time of pneumonia, with similarly timed NP bacterial profiles from their age-matched controls.

Methods: The study was nested within a South African birth cohort study, the Drakenstein Child Health Study. Nucleic acid was extracted from NP specimens from 8 children with pneumonia and 8 controls (together with the preceding NP specimens) using the automated QIAsymphony® SP instrument. Illumina MiSeq sequencing was performed targeting the V4 region of the bacterial 16S rRNA gene.

Results: Dominant phyla identified from NP specimens were Proteobacteria (85%), Firmicutes (7%), Bacteroidetes (3%), Fusobacteria (3%) and Actinobacteria (2%). The most abundant genera identified were Moraxella (60%), Haemophilus (17%) and Streptococcus (4%). The genus Haemophilus was more abundant in case specimens compared to specimens from controls, while the genus Moraxella was more abundant in control specimens. Proportions of the genus Streptococcus increased progressively in cases leading up to the time of pneumonia. However, differences between cases and controls were relatively small and need to be validated in a larger cohort.

Conclusion: This small pilot study of eight age-matched case-control pairs shows changes in the NP microbiota preceding pneumonia, however differences in profiles from cases and controls did not reach statistical significance. Larger sample sizes will be assessed in future.
Human immunodeficiency virus (HIV) is a Lentivirus of the Retroviridae family and is the causative agent of the acquired immunodeficiency syndrome (AIDS). HIV is currently characterized into HIV-1 and HIV-2 which differ in genomic structure and antigenic makeup. Subtype C is the most prevalent HIV-1 variant strain in South Africa and it accounts for majority of infections globally. The problem statement of this study is based on the fact that HIV/AIDS is the leading cause of death worldwide and the escalating number of death is also due to HIV related diseases especially in South Africa were not all people have access to high quality public health care. The high levels of people infected with HIV/AIDS in South Africa have major threats towards the developing economy of the country. There are many factors contributing to the high level of HIV infected people and its spread in South Africa such as the engagement of unsafe sex with HIV infected people more over with multiple partners, rape, migrant labour system and limited public awareness about HIV/AIDS. However the prevalence of HIV/AIDS in South Africa is associated with the high occurrence of drug resistant HIV strains which are reducing the effectiveness of ART therapy and the development of a vaccine. Therefore there is a constant need to identify these strains in order to monitor HIV in infected people especially the ART drug resistance HIV strains and production of an effective treatment. The aim of this study is to identify HIV-1 subtype C drug resistance causing mutation on pol, genes in HIV positive patients. The objectives of this study are to determine the protease and reverse transcriptase mutations by real-time PCR using genotype specific primers. From this study we expect to be able to identify different drug resistance strains of patients who are HIV positive.
Phenol degradation enhancement of Parent Acinetobacter strain V2 by a step-wise continuous acclimation process was investigated. At the end of eight months, 3 stable adapted strains, designated as R, G and Y, were developed with the sub-lethal concentration of phenol at 800, 1100 and 1400 mg/l respectively. All strains degraded phenol at their sub-lethal level within 24 hours, their growth rate increased as the acclimation process continued and retained their degradation properties even after storing at -80°C for more than 3 years. All adapted strains appeared coccoid with an ungranulated surface under electron microscope compared to typical rod-shaped parental strain V2. The adapted Y strain possessed superior degradation ability against other phenolic compounds. This study demonstrated the use of long term acclimation process to develop efficient and better pollutant degrading bacterial strains with potentials in industrial and environmental bioremediation.
Medicinal plants are rich in secondary metabolites thus making them known for their specific medicinal use. There is a low concentration of metabolites produced in wild plants and an attractive alternative to overcome this limitation is plant tissue culture techniques. *Agrobacterium rhizogenes* has been employed to genetically transform plant tissue thus allowing the higher production of metabolites. Tropane alkaloids are anticholinergic agents which holds a high pharmaceutical value. These phytochemicals are found in the wild plants of the *Solanaceae* family, however their overall yield is relatively low. Hence, this project was focused at using cell and tissue culture systems of *Datura stramonium* to increase the production of tropane alkaloids, specifically atropine and scopolamine.

The establishment of *D.stramonium in vitro* plant system required a source of sterile explants. A protocol utilising 30% sodium hypochlorite was optimized for sterilization and germination of seeds following micro-propagation on MS medium supplemented with 6-Benzylamine purine and Indole acetic Acid. The *in vitro* culture served as a source of explants for the induction of callus and hairy root cultures. Hairy root cultures were induced using *A.rhizogenes* 15834.

The cultures were bulked extraction and analysis of tropane alkaloids. Concentrated methanolic extracts from field leaves, roots, shoot, cell suspension and hairy root cultures were used for the quantitative analysis and comparison of atropine and scopolamine. Atropine and scopolamine eluted at 6.215 minutes and 4.600 minutes respectively. Callus cultures produced a higher yield of atropine and scopolamine at 7.5 µg.ml⁻¹ and 12.96 µg.ml⁻¹ as compared to hairy root cultures of 1.22 µg.ml⁻¹ and 1.81 µg.ml⁻¹.
S7.02 - PROTEOMIC CHARACTERISATION OF WINE YEASTS FOR THE EXPRESSION OF ARGINASES INVOLVED IN UREA FORMATION DURING FERMENTATION

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Ethyl carbamate (EC) is a chemical compound found in alcoholic beverages, implicated in toxicity and carcinogenicity. Ethyl carbamate is formed when urea, a by-product of fermentation, reacts with ethanol at higher temperatures. Small-scale winemaking trials (18 L), using commercial wine yeasts were performed in Sauvignon blanc and Cabernet Sauvignon grape must. Ion-exchange chromatography in conjunction with spectrophotometry was used to measure urea levels in wine. The yeast strain, Prise de Mousse (PdM) was the lowest urea producer in both Sauvignon blanc and Cabernet Sauvignon wines. The highest urea producing yeast strain varied for Cabernet Sauvignon and Sauvignon blanc wines, but in both cultivars exceeded 2 mg/L. Subsequently, these wines does not comply with the Canadian legal limit. Chemical analyses showed that all wines fermented to dryness and the sensory evaluations showed that none of the wines were negatively perceived by the judges. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed differential protein expression for the various yeast strains. The proteins of interest were therefore excised and characterised by using matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF) and will be investigated further for functionality.
The use of non-*Saccharomyces* in combination with *Saccharomyces cerevisiae* yeasts to add complexity and improve wine quality is increasing in popularity and therefore it is important to understand the potential impact on the secondary malolactic fermentation (MLF) carried out by lactic acid bacteria (LAB). The aims of this study were therefore to investigate the interactions between *Saccharomyces*, non-*Saccharomyces* yeasts and LAB, and the resulting impact on MLF and wine flavour. Shiraz wines were fermented with two commercial *Saccharomyces* yeasts as single inoculums and in combination with a non-*Saccharomyces* yeast, *Metschnikowia pulcherrima* and *Hanseniaspora uvarum*, respectively. Two different LAB species, *Oenococcus oeni* and *Lactobacillus plantarum*, were also evaluated in co- and sequential inoculations. Wines fermented with the non-*Saccharomyces* yeast combinations contained lower alcohol and glycerol levels than the reference wines. Malolactic fermentation completed faster in the wines fermented with non-*Saccharomyces* yeasts. Wine composition was also positively affected. Wines produced with the different non-*Saccharomyces* yeast combinations had significantly more “fresh vegetative” and “wood associated” aromas, and “body” than the reference wines. Wines where *O. oeni* was used to induce MLF were significantly different to wines where *Lb. plantarum* was used, and had less “berry” and “sweet associated” aromas. Yeast selection, LAB strain and time of MLF induction had a significant effect on the chemical and sensory properties of the wines.
S7.O4 - THE EFFECT OF CLOSANTEL ON FAECAL PHOSPHORUS, CALCIUM AND MAGNESIUM IN BOER GOATS GRAZED AT MOLELWANE FARM, MAFIKENG.

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The present study was conducted to investigate the effect of Closantel on faecal Phosphorus (P), Calcium (Ca) and Magnesium (Mg) in Boer goats grazed at Molelwane farm. Gastrointestinal nematodes (GIN) represent a major economic hurdle in ruminants system and anthelminths are estimated to account for 53% of the total costs of veterinary drugs worldwide (Diaz Lira et al, 2008). Twenty Boer goats were selected for the study and were divided into two groups of ten animals in each group; first treatment group which were drenched with Closantel at 1ml/kg body weight and control group which was not drenched. Faecal samples were collected for five months, from February to June. The mean helminth eggs per gram of faeces treatment group was reduced to zero in the last three months of the trial. In the control group, number of egg per gram increased. The treated goats had low mineral content in their faeces compared to those in control group, especially in the last three months of the trial due to zero level of infection, thus leading to high rate of absorption of minerals on treated goats because there was no disturbance in their gut.
In 2013, World Health Organization (WHO) recorded 1.5 million deaths and 9 million cases of tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtbc*). *Mtbc* secretes a large number of proteins into the surrounding environment and these play an important role in host-pathogen interactions as they influence the host cell response while facilitating invasion of the host. Secretory proteins are present in blood and other bodily fluids making them good candidates for drug discovery, vaccine design and diagnosis. Traditionally, *Mtbc* secretory protein studies were based on the analysis of culture filtrates. The availability of *Mtbc* whole genome sequence has encouraged in silico studies of Open Reading Frames (ORFs) to identify secretory/extracellular proteins. The present study aimed to identify *Mtbc* secretory proteins using a phage display vector specific for peptides whose DNA fragment encodes some signal peptide sequence (SPS) for secretion. A *Mtbc* phage library of ~1.72x10^6 clones was constructed from the virulent KZN605 genomic DNA fragments (200bp to 1500bp). Phage DNA isolated from 120 randomly selected phages was sequenced and blasted against *Mtbc* Comparative Database ([http://www.broadinstitute.org/](http://www.broadinstitute.org/)) in order to identify ORF’s. Ninety-eight distinct ORF’s were identified, of which 56 were *Mycobacterium*-specific proteins (MSSPs). MSSPs included 33 proteins encoding SPS, 13 membrane proteins, and 10 proteins without SPSs (such as PPEs, ESX-1 and other unknown proteins). MSSPs included conserved proteins required for virulence, host cell invasion, in vivo survival and active evasion of the host’s immune response. Potential TB drug targets, vaccine candidates and TB diagnostic biomarkers were also identified. Our findings support phage display as a powerful and appropriate in vitro technique for identification of useful *Mtbc* biomarkers.
The CSIR Biosciences are the leaders in fermentation process development and are often approached by Industry to develop new, or improve existing production processes. One such request required the improvement of sporulation efficiencies for a Bacillus isolate. The aim of the development work was to improve cell concentration to greater than $1 \times 10^{10}$ cells.mL$^{-1}$ while achieving a final sporulation efficiency of >80%. On commencement of the project, the isolate was unable to sporulate.

The study entailed the evaluation of reduced protein content on the cell concentration and sporulation efficiency of the test isolate.

The Bacillus isolate was initially culture on an existing CSIR proprietary recipe which resulted in a high cell concentration ($\sim 4 \times 10^{10}$ cells.mL$^{-1}$), however, no sporulation was observed during this study. Experiments were then designed to reduce total protein content in fermentation by 43, 83, 87, 91, 94 and 95%. The results demonstrated that a reduction in protein concentration yielded improved sporulation efficiencies. Unfortunately this improved efficiency resulted in an inverse effect on cell concentration, with the best performing recipe on sporulation efficiency (85%) realizing a 34% reduction in final cell concentration.

Although there was a 34% reduction in cell concentration the target of $1 \times 10^{10}$ Cells.mL$^{-1}$ was still achieved. The final product is stabilized Bacillus spores; therefore the improved sporulation efficiency is of utmost importance. The improvement has taken the saleable product from 0 spores.mL$^{-1}$ to $\sim 1.4 \times 10^{10}$ spore.mL$^{-1}$. 
Soil transmitted helminths (STHs), like *Ascaris*, *Trichuris* and hookworms are a major public concern worldwide. Infection mainly occurs through environmental exposure (water, soil, contaminated crops like lettuce and cabbage etc). In endemic areas wastewater can contain up to ~3000 STH ova/L. Exposure to wastewater, contaminated surface water or faeces/sludge will result in higher risk of infection.

A comparative epidemiological study conducted in Ghana, showed an association between exposure to STHs eggs in wastewater/soil and actual infection in 165 farmers and family members (control group 100 non-farmers). All participants were dewormed and confirmed negative with stool samples after a week. Stool sampling was repeated after three months. Multiple regression (*GraphPad Prism* 6) and odds of infection (Odds Ratio, Altman, 1991) was used. Egg loads in wastewater/soil and actual infection loads was determined to 0.19 (r-value) for irrigation water and 0.54 (r-value) for soil, indicating a higher association between soil and infections, probably due to accumulation of STHs eggs over time. Farmers were three times most likely to be infected with both *Ascaris* and hookworm, especially in the wet season (OR 3.99 (95% CI; 1.15-13.86) for *Ascaris* and 3.07 (95% CI; 0.87-10.82) for hookworm).

Several methods for the detection and quantification of STHs eggs in environmental samples are currently in use which makes comparative risk estimation difficult. Through an ongoing Gates project a harmonization and refinement of recovery rates, accuracy and consistency of methods are ongoing between Senegal, India, Mexico and South Africa (UKZN and DUT) to assess the methodological accuracy for future risk estimations and in depth epidemiological studies.
The presence of Bt (Bacillus thuringiensis) toxin gene in maize (Zea mays L.) plants might change the structure of plants associated microbes. Although there are benefits assigned to Bt toxin producing maize plants, there is less information available on potential roles of endophytes linked to them. Endophytes are microorganisms that dwell within the robust plant tissues by having a beneficial association with their host. Our study aims at identifying the diversity and potential roles of cultivable endophytes associated with transgenic (Bt) and non-transgenic (Non-Bt) maize plants. Twenty isolates were obtained from the shoots of transgenic (Bt MON810) and eleven from its parental (Non-Bt) maize. The isolates were screened for capacity to solubilise phosphate, nitrogen fixation, antifungal activity and IAA production. The genera found to be associated with Bt maize were identified as Bacillus, Pantoea, Stenotrophomonas, Acinetobacter, Yersinia and Serratia. Non-Bt maize had the same genera as Bt except the presence of the genus Escherichia and absence of Serratia and Pantoea genera. All strains were able to fix nitrogen and IAA production was detected in 16 strains with Pantoea sp. (Bt 2L) identified to produce the highest concentration. Furthermore, Yersinia sp. (NBt 10H*; NBt 3L*), Bacillus sp. (NBt 10C2) and Serratia sp. (Bt 1C) had high percentage inhibition against the kernel pathogen, F.verticillioides. Acinetobacter sp. (Bt 9C**) obtained from the seeds was identified to be the highest phosphate solubilisers with 51% efficiency. The results shows that maize endophytes have potential to participate in nutrient cycling hence, highlighting their importance in agriculture.
Alteration index three (AI3), which calculates the balances between three microbially-secreted enzymes, potentially enables differences between soils due to contrasting management practices to be quantified in relative terms. Internationally, testing has shown that AI3 could distinguished between soils that ranged from mine spoil to forest. Locally, and of greater relevance to the South African apple industry, AI3 may prove to be a useful indicator of soil health. The ability of AI3 to distinguish between contrasting soil management regimes, and to reflect soil chemical status and apple tree performance, was tested in trials involving mulch, fumigation, and cover crop treatments. AI3 indices were generated using the formula by Puglisi et al. (2006). Tree row soils samples were taken from different depth intervals within a year of treatment application. Lower AI3 indices, and by inference, better soil health, were associated with mulched compared to bare soils, confirming the known positive contribution of mulching to general soil health. Lower AI3 indices associated with control compared to fumigated soils support the generalized view that fumigation suppresses microbial-mediated functions. AI3 detected differences between cover crop treatments; cover crops with bio-fumigant properties had better overall AI3 scores than the control. Lower AI3 indices were generally associated with top- than subsoils, reflecting gradients in mineralizable substrates across soil layers. Correlations between AI3 and soil chemical and tree parameters were inconclusive, which is probably due to the slower chemical and tree reactions to treatments. These findings attest to the usefulness of AI3 as an indicator of soil health in local apple orchards.
Bacillus species have been known to antagonize and inhibit the growth of various plant pathogens. The aim of this study is to analyze the mineral composition of tomato to which Bacillus isolates were applied as biocontrol agent whose relatedness/diversity has been profiled using Random Amplification of Polymorphic DNA (RAPD). Eleven Bacillus isolates were characterized using RAPD primers A9B7, OPH19 and S4. Four of them randomly selected namely, B. amyloliquefaciens, B. cereus, B. pumilus and B. subtilis were used as antagonists against Fusarium wilt pathogen of tomato. From the dendrograms drawn, only B. pumilus exhibited clear-cut difference compared to the other Bacillus isolates. Harvested tomatoes were analyzed for mineral composition using Energy-dispersive X-ray (EDX) 720. Plants treated with B. cereus had highest fresh and dry mass of tomato plant of 6.73 g and 1.21 g respectively compared with the control of 2.82 g and 0.19 g respectively. Plants treated with B. amyloliquefaciens had significantly bigger tomatoes with dry weight of 12.61% compared to control (7.47%). Tomatoes from treatment with B. amyloliquefaciens and B. subtilis had 3.88 mg/g of Potassium compared to control (1.80 mg/g) and 0.73 mg/g of Calcium compared to control (0.18 mg/g) respectively. Tomatoes treated with B. amyloliquefaciens and B. pumilus had 0.06 mg/g of Copper and 0.01 mg/g of Manganese respectively and both treatments individually had tomatoes with 0.01 mg/g of Rubidium. These results suggest that these Bacillus isolates can be used to control Fusarium wilt and promote quality of tomato while their consortium can be researched.
S10.02 - METABOLIC FLUXES IN KLUYVEROMYCES MARXIANUS: REGULATION AND COMPARISON WITH OTHER YEASTS

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High-temperature, anoxic conditions are ideal for large-scale biofuel production, two traits that make the yeast *Kluyveromyces marxianus* attractive as a host for metabolic engineering. Such conditions are known to induce oxidative stress. A recent study suggested that increased NADPH production may be part of the response in overcoming these adverse conditions. We explore the metabolic fluxes in central metabolism using 13C-Metabolic Flux Analysis under different aeration regimes and temperatures to resolve the intracellular fluxes that contribute to redox metabolism. Distinction is made between fluxes under genetic and kinetic level regulation by combining fluxes with RNA-seq data. Fluxes in this yeast are compared with those of other species and evolutionary trends in yeast central metabolism are shown. Finally, we evaluate the potential of this yeast for fully anaerobic growth along with a number of other species and attempt to identify genes responsible for anaerobic growth in a variety of species using comparative genomics.
Members of the class *Actinobacteria* are widely known for their ability to produce bio-active compounds. Of the almost 60-80 000 microbial natural products identified to date, approximately 20 000 are produced by actinobacterial strains. Even though genome sequencing has allowed for the identification of new and interesting biosynthetic gene clusters in actinobacteria, the rate of discovery of new compounds for development of pharmaceutical products remains low. Actinobacterial genomes typically range from 4.5 to 10 Mbp in size and therefore represent a rich potential resource for novel bio-active compounds. Understanding the environment from which an actinobacterium has been isolated as well as knowing its phylogenetic position within the class *Actinobacteria*, allows for the prediction of their potential to produce novel bio-active compounds.

In this study, the genome sequences of *Streptomyces polyantibioticus* SPR\(^T\) and *Streptomyces pharetrae* CZA14\(^T\) were analysed for the presence of biosynthetic gene clusters. An analysis of the genomes using antiSMASH, revealed the presence of 56 biosynthetic gene clusters in the SPR\(^T\) genome and 55 clusters in the CZA14\(^T\) genome. Various cultivation conditions allowed for access to the bio-active compounds of interest. The antimicrobial activities of crude extracts were tested against a range of known pathogenic test strains to determine their potential application. In addition, extracts were analysed by thin layer chromatography and bio-active spots extracted and analysed by liquid chromatography mass spectrometry. The online resource, DoBISCUIT, allowed for the results obtained from the chromatography analyses to be linked to the biosynthetic potential information in the genome sequences.
Bio-ethanol, produced from lignocellulosic agricultural waste, has the potential to replace fossil based transportation fuels. Enzymes required to hydrolyse the lignocellulosic fraction of agricultural waste to simple sugars are considered to be the major contributor to the high ethanol production cost. In this study we evaluate the potential of using yeast hybridization with a consolidated bioprocessing (CBP) approach as a means to reduce or eliminate the addition of cellulytic and hemi-cellulytic enzymes to the ethanol production process. High cellobiohydrolase I secreting progeny was isolated after hybridizing industrial bio-ethanol yeast strain \textit{S. cerevisiae} M0341 and laboratory strain \textit{S. cerevisiae} Y294. These strains were evaluated on their ability to secrete other relevant recombinant hydrolase enzymes for CBP-based ethanol production, namely β-glucosidase I, Xylanase II, Endo-glucanase III, and Cellobiohydrolase I. A total of seven \textit{S. cerevisiae} host strains were constructed, two parental strains \textit{S. cerevisiae} M0341 and \textit{S. cerevisiae} Y294 and their hybrid strains \textit{S. cerevisiae} H3M1, H3M28, H3H29, H3K27 and H3O23 including five episomal plasmids namely, each containing a different reporter enzyme transformed into these strains. Enzyme activity assays indicate that both the xylanase and endo-glucanase was not secreted at higher titers compared to the parental strains. M28_Cel7A was found to be the best secretor of cel7A (Cellobiohydrolase I), however this phenomenon also imposed a significant metabolic burden on the yeast. The data indicate that the high enzyme secretion ability in this study is specific to cellobiohydrolase I.
**S10.O5 - EFFICIENT EXTRACELLULAR EXPRESSION OF α-AMYLASE FROM BACILLUS LICHENIFORMIS AS08E IN ESCHERICHIA COLI USING BACILLUS SIGNAL PEPTIDE**

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*Escherichia coli* is regarded as 'protein factories' for the heterologous expression of recombinant protein, however, sometimes it cannot express successfully protein containing complex disulfide bonds or high molecular protein (>60kDa). Therefore, the present investigation was aimed to express the active recombinant α-amylase from *Bacillus licheniformis* AS08E in *E. coli* using native signal peptide from *Bacillus*. The gene encoding α-amylase (Blamy-I) from *B. licheniformis* AS08E was isolated and PCR amplified using *Bacillus* gene specific primers. The PCR amplified product was cloned into pET28a expression vector and over-expressed in *E. coli*. The native Blamy-1 consists of 483 amino acids with predicted molecular weight of 55.3 kDa and a pI of 6.05. The entire amino acid sequence of Blamy-I gene is 99% similar with the amylase gene from *B. licheniformis* CICC 10181 and contain *B. licheniformis* signal peptide. Further, the signal peptide from Blamy-1 gene was explored to facilitate the extracellular secretion of Blamy-1 from *E. coli* into the culture media and it was found that it can efficiently express the active form of recombinant protein. However, the secretion of recombinant protein was very limited. Therefore, to maximize the extracellular secretion of Blamy-I from *E. coli* into the culture media, the culture parameters affecting extracellular expression of recombinant protein were optimized using response surface methodology. Under the optimized condition, the Blamy-I production in culture media was about seven times higher than the non-optimized condition. Thus, the present approach proves to be efficient for the extracellular expression of active recombinant enzyme in *E. coli*. 
Cassava serves as a staple food crop for over 800 million people and is particularly important for the sustainable livelihoods for resource-poor farmers in sub-Saharan Africa. It is also used in biofuel, animal feed and industrial raw material production. South African cassava mosaic virus (SACMV) belongs to the family Geminiviridae and is one of the causal agents of cassava mosaic disease (CMD). MicroRNAs (miRNAs) comprise a large group of 21 – 24 nt RNA molecules that play a crucial role in stress response in plants, including biotic stress caused by viral infection. Viruses however can interfere with and exploit the silencing-based regulatory networks, causing the deregulation of miRNAs. This study aimed to understand the regulation of miRNAs in tolerant (TME3) and susceptible (T200) cassava landraces infected with SACMV. Next-generation sequencing was used for analysing small RNA libraries from infected and non-infected cassava leaf tissue collected at 12, 32 and 67dpi (days post-inoculation), and normalized against mock inoculated samples. A full repertoire of cassava miRNAs was characterized, which included conserved and novel cassava-specific families. The total number of differentially expressed miRNAs across all three time-points was 209 and 221 miRNAs, belonging to 34 families, in TME3 and T200 infected plants, respectively. A high number (51 and 109 miRNAs in TME3 and T200, respectively) were significantly altered at 32 dpi when plants showed severe symptoms. Notably, in T200 the 109 significantly altered miRNAs were all upregulated. Endogenous targets were predicted in the cassava genome for many of the identified miRNA families. Interestingly, some of the miRNA families (miR162, miR168 and miR403) that we significantly deregulated in both T200 and TME3 upon SACMV infection were shown to target proteins (DCL1, AGO1 and AGO2) that play important roles in the RNA silencing pathway. The results of this study provide insights into miRNA-mediated SACMV cassava interactions and may provide novel targets for control strategies aimed at developing a CMD-resistance cassava cultivar.
**S11.O3 - ISOLATION OF ANTIBACTERIAL COMPOUNDS FROM SENNA ITALICA LEAVES AGAINST BACTERIA WHICH INFECT WOUNDS**

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*Senña italica* leaves were collected from Zebediela region of Limpopo during the summer season. Preliminary studies carried out on the leave extracts showed inhibitory activity against *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus*. Crude extracts for isolation were prepared using exhaustive extraction with hexane, dichloromethane (DCM), acetone and methanol. The extracts were analysed for phytochemical analysis and biological activities. The DCM extracts showed the most antibacterial activity on the bioautograms and were therefore selected for the isolation of antibacterial compounds. Column chromatography was used to isolate active compounds which were analysed using Thin Layer chromatography for phytochemical analysis, bioautography and antioxidant activity. The isolate was purified through preparative Thin Layer Chromatography using 100% chloroform as the eluent. The purified isolate was sent to the Centre for Scientific and Industrial Research (CSIR) for analysis on \(^1\)H and \(^{13}\)C nuclear magnetic resonance (NMR) and Mass Spectrometry (MS) for structural analysis. This study shows that there is a biologically active compound belonging to the alkane group which may be unique. Although the compound isolated was not identified due to a contaminant, further work is required to identify the specific alkane and to determine if it is indeed unique and a good candidate for the treatment of infected wounds.
S11.O4 - SMALL RNA AND METHYLATION DEFENCE RESPONSES IN SUSCEPTIBLE AND TOLERANT LANDRACES OF CASSAVA INFECTED WITH SOUTH AFRICAN CASSAVA MOSAIC VIRUS

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There is strong evidence indicating that innate immunity and RNA silencing in plants are closely linked. Virus-derived small interfering RNAs (vsRNAs) have been implicated in recovery or symptom remission in some natural geminivirus-host interactions. Transcriptional gene silencing (TGS) (24 nt siRNAs) and post transcriptional gene silencing (PTGS) (21-22 nt) of viral DNA and mRNA, respectively, is also a consequence of geminivirus infection. In this deep sequencing study, we compared the response to South African cassava mosaic virus (SACMV) of cassava TME3 which shows symptom recovery at 67 days post infection (dpi) and a tolerant phenotype, and T200, a highly susceptible landrace. While vsRNAs of 21, 22 and 24 size classes targeted the entire SACMV DNA-A and DNA-B genome components in T200 and TME3, there was no clear evidence for a strong correlation between TGS and recovery in TME3 at 67 dpi as the increase in methylation-associated 24 nt vsRNAs was low (9\% of total vsRNA populations) and methylation not detected by bisulfite sequencing. All vsRNA size classes were significantly more highly represented in susceptible T200 at 32 and 67 dpi compared with tolerant TME3, suggesting that in T200 siRNAs accumulate as they fail to target SACMV mRNA post-RISC processing, leading to susceptibility. Novel endogenous tasiRNAs were identified in T200 and TME3 and different patterns in terms of targets and population counts were observed. Interestingly, a NBS-LRR resistance protein RGH1) was shown to be targeted by a tasiRNA in T200 and TME3. We demonstrate that PTGS is a basal immune response in both T200 and TME3, but conclude that it is not sufficient to provide resistance in T200, and plays a minor role in recovery in TME3. Other intrinsic features of geminiviral genomes, and their molecular interactions with different host species, involving miRNA and tasiRNA-mediated transcriptional responses, as well as a fluctuating balance between silencing and silencing suppression, are likely to influence the efficacy of virus-induced PTGS and play a role in natural resistance.
Aerobic endospore-forming bacteria (AEFB) are recognized as promising candidates for biological control of powdery mildew of cucurbits. A study was undertaken whereby AEFB were isolated from the phylloplane of cucurbits and screened as potential antagonists using a multifaceted approach. Isolates were obtained from leaf material sourced from eight locations in the greater Msunduzi, KZN region. Antifungal activity was assessed with dual-culture bioassays using surrogate phytopathogens in place of obligately biotrophic Podosphaera spp.. Dereplication amongst antagonistic AEFB was undertaken using ITS-PCR and RAPD-PCR genotyping methods. Fourteen fingerprint profiles were distinguished by RAPD-PCR, and profile representatives underwent phylogenetic analysis of 16S rRNA and gyrA gene fragments. Several distinct clusters were detected with the gyrA sequences providing greater strain-level sequence heterogeneity than 16S rRNA. Sequence comparisons to the GenBank database revealed similarities to several plant-associated B. amyloliquefaciens subsp. plantarum and B. subtilis strains. Identification of selected isolates using MALDI-TOF-MS and the Bruker BDAL Biotyper database was evaluated. Twenty percent of isolates achieved species level identifications with acceptable confidence levels. Mass spectra profile dendograms using Bruker Biotyper and online clustering tool SPECLUST achieved clustering of related isolates. Lipopeptide compound production was determined using PCR-based screening for selected lipopeptide gene markers and MALDI-TOF-MS analysis of extracts. Isolates produced multiple lipopeptide homologues; including those of surfactin, iturin, and fengycin. Disparities between PCR and MALDI-TOF-MS data suggest that some PCR primers showed limited specificity amongst the AEFB strains screened. Based on the overall findings, nine isolates proceeded to screening against Podosphaera spp. in vivo.
Ammoniacal nitrogen in domestic wastewater is a potent agent of eutrophication within receiving water bodies. Conventional ammonia removal is an energy intensive process that relies on the synergistic action of two spatially separated and metabolically distinct bacterial populations. Conversely, the Anammox bacterial group is able to convert ammonia to nitrogen gas using a single metabolic pathway under anoxic conditions. This technology may thus present a viable alternative to conventional nitrogen removal processes due to comparatively lower energy requirements, reduced cost and smaller waste footprints. In this study, a bench scale continuous stirred tank reactor was constructed to selectively enrich for a microbial consortium with a high affinity for NH$_3^+$ and low affinity for oxygen. Ammonia and nitrite removal were observed from the day 6 of enrichment, exhibiting rapid acclimatisation of the seed inoculum to the high ammonia levels. Stable ammonia removal was achieved after 358 days of operation of the reactor with an ammonia conversion rate of ± 96% in 24 h (influent ammonia at 150mg/L). Additionally, ammonia removal occurred with the expected reduction in NO$_2^-$ (>99%), but also the concomitant production of NO$_3^-$ from ~45mg/L (avg.) on days 1-135 to ±137mg/L at day 136. These results together with 16S rRNA based PCR screening revealed the co-existence of nitrifiers and anammox populations in the reactor. After 360 days of enrichment, the stable co-population of anammox bacteria and nitrifiers in a single reactor at low DO concentrations infers that these populations serve to stabilize each other in the presence of the high ammonia load and act synergistically to degrade the influent ammonia.
Terrestrial systems are important carbon reservoirs and are currently undergoing extensive changes due to elevated greenhouse gas emissions. The effects of this change may have consequences on the relationship between biodiversity and ecosystem function (biogeochemical cycling), particularly on microbial communities who are major drivers of these cycles. Antarctica soils lack higher life forms (i.e. plants), are microbially-driven, and may be sensitive model systems for understanding the effects of global change processes (such as temperature fluctuations) on biogeochemical cycles. To clarify the relationship between biodiversity and ecosystem function, we constructed Antarctic soil microcosms and applied temperature fluctuations over a 30 day period. The applied temperatures included two stable controls (0°C, 15°C) and one test increasing by 1.5°C increments per day until a plateau of 15°C was reached. We used 16S rRNA gene amplicon sequencing and enzymatic assays to assess microbial community structure and function. The enzyme assays performed included alkaline phosphatase, chitinase, leucine aminopeptidase, β-glucosidase, β-xylosidase and phenol oxidase/peroxidase. Preliminary results from enzymatic assays indicate that a greater functional activity is observed at higher temperatures. Sequencing data will allow us to elucidate the phyla implicated in these functional processes. Taken together, the results of this study will indicate whether there is functional redundancy among microbial communities, or whether a few dominant phyla are responsible for driving functional processes.
S12.04 – DETECTION OF CARBAPEM RESISTANT KLEBSIELLA PNEUMONIAE IN A MUNICIPAL WASTEWATER TREATMENT PLANT IN STELLENBOSCH, SOUTH AFRICA

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The effluents from urban wastewater treatment plants (WWTP) are among the main anthropogenic sources of antibiotics, antibiotic resistant genes and antibiotic resistant bacteria in the environment. The primary aim of this study was thus to detect carbapenem resistant Klebsiella pneumoniae strains at various points of the Stellenbosch WWTP. Wastewater samples were then collected for three cycles from four sampling sites, namely, the influent point, aeration tank, secondary settling tank and effluent point. Klebsiella pneumoniae isolation was accomplished by spread plating the dilution factors $10^{-3}$ and $10^{-4}$ from each respective sampling site onto HiCrome Klebsiella Selective Agar (HKSA) in duplicate. From the three sampling cycles and the various sampling points, 20 K. pneumoniae isolates were positively identified using 16S rRNA PCR, Matrix-assisted laser desorption/ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) and the VITEK 2 bacterial identification system. Six of these K. pneumoniae isolates were found to have the resistant ß-lactam Klebsiella pneumoniae carbapenemases (blaKPC) gene on genomic or plasmid DNA using PCR. The VITEK 2 antibiotic susceptibility system then showed that all six K. pneumoniae isolates were resistant to the carbapenem class of antibiotics, with two of the isolates obtained from the effluent point of the WWTP. This result indicates that antibiotic resistant bacteria are being released in the effluent of the Stellenbosch Waterworks, which flows into the Eerste River. Water from this river system is then used by neighbouring agricultural farms for irrigation purposes. This raises significant public health concerns and may potentially compromise the export of this produce.
Microorganisms isolated from hot springs (Bacillus and Bacillus-related bacteria) have proved valuable in several applications including bioremediation of water. This work describes the first investigation of cultured microorganisms from hot springs in South Africa with temperature ranging from 45°C to 68°C and pH of 7 – 9. Further characterisation will include screening for potentially important enzymes useful in water bioremediation. Bacterial isolations were made from water and sediment samples on four different media (nutrient agar, minimal 10% Luria agar, potato dextrose agar, actinobacter agar) and incubated aerobically 55°C for thermophiles, and 37°C for mesophiles. The majority of isolations were made on minimal and nutrient media. Following polymerase chain reaction (PCR) of DNA with universal bacterial primers 27F and 1472R, the 16S rDNA gene was sequenced for identification using a BLAST search on Genbank, and phylogeny studies. Forty-three isolates were identified within the phylum Firmicutes, in two main families Bacillaceae (Bacillus and Anoxybacillus) and Paenibacillaceae (Aneuribacillus and Brevibacillus) as gram-positive endospore-forming rod-shaped bacteria. The largest group (n=27) were related to Bacillus licheniformis or Bacillus subtilis. Two minor groups were related to Anoxybacillus species (n=8) and Brevibacillus species (n=5). Three single isolates were tentatively identified as Bacillus pumilis, Bacillus panaciterrae and Aneuribacillus sp respectively. All Anoxybacillus spp, 81% B subtilis/B licheniformis spp and 40% Brevibacillus spp were isolated at 55°C. One alkali-thermophile isolate from Siloam hot springs was found to optimally grow at a pH of 10 and temperature of 50°C. There was no clear association of phylogeny groupings with geographical location.
Marine microbial natural products (NPs) are recognised as an important sustainable source of novel drug candidates for the treatment of drug-resistant infections that are a global health crisis. This study aimed to discover novel NPs from bacteria associated with marine invertebrate species endemic to the South African coast, including a sponge *Spongia* (*Spongia*) sp. and a tunicate, *Pseudodistoma africanum*. Bacteria associated with the invertebrate species were isolated and screened for antimicrobial activity against a panel of indicator strains including a multi-drug resistant *E. coli* strain. Anti-bacterial activity was detected in 6.1% and 4% of bacterial isolates from the sponge and tunicate isolates respectively. One of the isolates, PE8-15, belonging to the genus *Bacillus* was selected for further investigation due to its potent activity against a range of microbial pathogens. Genome sequencing revealed 10 secondary metabolite pathways including bacteriocins (5), non-ribosomal peptides (NRPS) (3), siderophore (1) and a terpene pathway. One of the hybrid NRPS-PKS pathways, exhibiting low sequence identity (<65%) to reference sequences in the NCBI database, was predicted to encode a novel lipopeptide. Lipopeptides are an important class of compounds with a range of industrial applications in the pharmaceutical, cosmetic as well as food industry. Furthermore, this operon was hypothesised to encode the antifungal activity presented by PE8-15. Transcriptome analysis confirmed differential expression of the hybrid pathway which correlated to the conditions under which anti-fungal activity was observed. We are currently elucidating the lipopeptide chemical structure using LC-MS and NMR.
Mining has been South Africa’s mainstay of economy for over 120 years yet it results in the production of large volumes of acid mine drainage (AMD) which contaminates surface/ground water with high concentrations of heavy metals and radionuclides. One of the most harmful and persistent metal contaminants present in the environment is lead (Pb). The consumption of lead even in minute concentrations causes several detrimental health impacts. *Cupriavidus metallidurans* CH34 facilitates defence against intracellularly accumulated lead ions (Pb²⁺) through the activation of several genes encoded by the operon *pbrUTRABCD*. Amongst the proteins that play a role in Pb resistance, the protein PbrD functions as a metallo-chaperone that binds and sequesters Pb²⁺ within the cell, a process which can be exploited in the bioremediation of lead contaminated water. The study aims to establish whether recombinantly expressed PbrD maintains its ability to bind/capture Pb²⁺, *in vitro* for downstream applications. The complete gene sequence encoding PbrD was used as a template to synthesize the gene for cloning into the pET32 Xa/Lic vector. Recombinant PbrD was overexpressed in BL21 (DE3) *Escherichia coli* cells and confirmed by western blots using an S-tag antibody. The protein was purified to native form using S Tag™ purification kit and cleaved from the fusion tag using Factor Xa. The purified PbrD protein was further incubated with 1 mg/L, 10 mg/L and 100 mg/L Pb(NO₃)₂ for 24 and 48 hours and the concentration of Pb²⁺ bound to the protein was determined indirectly using inductively coupled plasma optical emission spectrometry (ICPOES).
The use of rivers for recreational and domestic practices makes it imperative to scrutinize the water quality circulating within the surrounding communities. Pathogens in the Umhlangane River were monitored at five points from October 2013 to September 2014. Indicator bacterial populations, physico-chemical properties, and coliphage populations were determined according to standard protocols. Tangential flow filtration was used to concentrate virus populations from the river water. Virus-like particle (VLPs) counts and viral morphology were determined using epifluorescence and transmission electron microscopy (TEM), respectively. Viral infectivity was assessed using cytopathic effect (CPE). The detection of some virus populations was determined by nested-PCR. An increase in the amount of chemical pollutants entering the water would allow for the high chemical oxygen demand, biological oxygen demand and the changing electrical conductivity and total dissolved solid levels. High E. coli, total and faecal coliforms could be attributed to faecal contamination entering the catchment. Canonical correspondence analysis suggests strong variance between the physico-chemical and bacterial data and a close relation between the bacterial and phage communities. Direct VLP counts were substantially lower than the plaques produced by the coliphages. Morphological changes of HEK293, Vero and Hep-G2 cell lines produced positive CPE for the viral concentrates. Apart from visualization of bacteriophages belonging to Caudovirales, presumptive Picornaviridae, Adenoviridae, Herpesviridae, Coronaviridae, Polyomaviridae and Reoviridae VLPs were visualized. Nested-PCR revealed adenovirus, polyomavirus and hepatitis A and C viruses in the Umhlangane River. The present study highlights the importance of routine environmental surveillance of microbial pathogens, particularly viruses in freshwater resources.
Several studies have assessed water quality in some of the rivers in the North West Province. A recent study also isolated yeast species from surface water sources. Some of these species are pathogenic and may cause mucosal to life-threatening infections. Antifungal drugs such as azoles are used to treat yeast infections. Prophylactic and continuous exposure of aquatic yeast species to sub-therapeutic levels of antifungal agents has resulted in yeasts becoming resistant to antifungal agents. The study aimed (i) to determine yeast levels and association with water quality (ii) antifungal resistance patterns in two rivers in the North West Province. Physical and chemical parameters were measured on-site and in the laboratory. Yeasts were enumerated using yeast-malt-extract (YM) agar. Disc diffusion antifungal susceptibility tests were conducted on isolated yeasts. Physico-chemical parameters of the water were within target water quality range (TWQR) for livestock farming but in most sampling sites out of range for irrigation use. pH, temperature, TDS, COD, NO₃⁻ and PO₄³⁻ levels ranged from 7.47 to 8.88, 14.2°C to 25.8°C, 456 to 970 mg/L, 0 to 103 mg/L, 0 to 7.8 mg/L and 1.07 to 2.07 mg/L, respectively. Yeasts levels ranged between 160-673cfu/L and 10-337cfu/L for room temperature and 37°C. All yeasts isolated were non-pigmented ascomycetous yeasts. Majority of yeast isolates (96%) were resistant to fluconazole, the most used antifungal agent to treat mycotic infections. All yeast isolates were resistant to metronidazole and flucytosine. Such antifungal resistance patterns amongst yeasts from environmental water sources that are used by local communities is a major health concern.

**Keywords:** Surface water resources, yeasts, water quality, antifungal susceptibility tests
S14.O2 – INFLUENCE OF PSEUDOMONAS AERUGINOSA ON EICOSANOID PRODUCTION BY CANDIDA ALBICANS

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Abstract

Pseudomonas aeruginosa and Candida albicans are opportunistic pathogens causing a range of infections in a nosocomial setting as well as in immunocompromised individuals. Their complex bidirectional interaction in co-infection has become increasingly clear. Their ability to prompt life threatening infections is due to their ability to form resistant biofilms as well as the manipulation of host immunity due to the production of immunomodulatory eicosanoids during infection. These eicosanoids are enzymatic products of arachidonic acid (AA), liberated from the host phospholipids during infection. Among others, the production of a secreted 15-lipoxygenase by P. aeruginosa as well as pyocyanin are potent virulence factors, mediating the production of 15-hydroxyeicosatetraenoic acid (15-HETE) from exogenous AA and reactive oxygen species generation due to pyocyanin. Candida albicans also possesses a range of virulence factors, including the ability to switch between yeast and hyphal morphology, the formation of biofilms as well as production of immune modulating prostaglandin E2 (PGE2) from exogenous AA. Since co-infection by these organisms in cystic fibrosis patients often occurs, it is important to gain a better understanding of the different levels of interaction between these organisms. This study evaluated the effect of P. aeruginosa on C. albicans biofilm eicosanoid production including PGE2, Prostaglandin F2α and 15-HETE through the use of enzyme linked immunosorbent assay (ELISA) and LC-MS/MS. The combination of virulence factors that P. aeruginosa maintains affected the production of eicosanoids by C. albicans in an in vitro dual species biofilm. Alteration of the eicosanoid profile of C. albicans and P. aeruginosa may have drastic effects on the virulence of these pathogens and their ability to infect and colonize hosts. This is particularly imperative in the case of cystic fibrosis as the host shows altered eicosanoid and inflammatory profiles.

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Keywords: Pseudomonas aeruginosa, Candida albicans, biofilms, eicosanoids
Antibiotic resistance spread in South African aquatic systems is a health risk to both humans and animals. Traditional culture-based methods for detection of antibiotic resistant bacteria and genes do not provide a full picture of resistance in the environment since 99% of bacteria cannot be cultivated. Metagenomics is a culture-independent method for the characterization of bacteria and can be employed in order to identify known and novel antibiotic resistant genes. Identifying novel antibiotic resistant genes will lead to better understanding of the mechanisms inducing resistance and ultimately monitoring gene spread. It has become more apparent that functional metagenomics is the most effective method to identify known and novel antibiotic resistant genes in uncultivable bacteria. Functional metagenomics does not require any prior knowledge of DNA composition of organisms. This method entails direct cloning of fragmented genomic DNA into an appropriate cloning vector, transformation of an appropriate host and expression of antibiotic resistant genes. Antibiotic resistant genes are identified following a screening process, isolated and sequenced in order to determine novelty when compared to online databases. The aim of this study is to evaluate the cloning protocols for functional metagenomic studies. This will be achieved by evaluating various DNA plasmid vectors for the efficiency of ligation of genomic DNA fragments that were isolated directly from sediments and bulkwater. The recombinant plasmids will be transformed into *Escherichia coli* JM109 cells and after a screening process determine which bacterial cells contain an antibiotic resistance genes. The genes will be sequenced followed by submission to an online database.

Key words: Antibiotic resistance, metagenomics, functional metagenomics, cloning.
The development of techniques for the synthesis of nanoparticles of well-defined size, shape and composition is a challenge and an important area of research in nanotechnology. Many microorganisms have the ability to produce inorganic nanostructures and metal nanoparticles with properties similar to chemically synthesized materials and are a good alternative approach to chemical synthesis. In the present study, extracellular synthesis of gold nanoparticles (AuNPs) in the presence of fungal extracts has been successfully demonstrated. The size and shape of gold nanoparticles could be manipulated by alteration of key growth parameters (Temperature, pH, incubation period, and sodium citrate concentrations) and reaction conditions (Supernatant: HAuCl₄). A colour change from yellow to violet-blue after ~72h of reaction was an indicator of AuNP production and confirmed by an absorbance peak at ~530 nm using UV-visible spectroscopy. The AuNPs were characterized by Transmission electron microscopy (TEM) and Fourier transform infrared (FTIR). TEM images revealed spherical, triangular, rod-shaped, and irregularly shaped AuNPs with indefinite morphology ranging between 3-460 nm. The most promising results were obtained when the fungus was grown at pH 3, 40ºC and the best parameters for AuNP synthesis were pH 3, 32ºC, 40 h, 5 mM sodium citrate concentrations and ratio of 1:100 Supernatant: HAuCl₄. The AuNPs were monodisperse, spherical 3-53 nm in size. FTIR spectrum showed the presence of bonds due to O-H stretching (around ~3,430 cm⁻¹), indicating the presence of proteins and other organic residues, which might have been produced extracellularly during the growth of the fungus. This study represents an important advancement in the use of fungal enzymes for the biosynthesis of highly stable gold nanoparticles by a greener approach and this proposed mechanistic principal might serve as a set strategy for the synthesis of nanostructures with desired morphology and can be amenable for large scale commercial production and technical applications.
Ethanol fermentation is one of the most important biotechnological processes. During this process, carbon dioxide (CO₂) and ethanol are produced. This process is used widely in the industry for the production of for example bread and alcoholic beverages. During fermentation in microorganisms, CO₂ is released vigorously into the environment. The expectation is therefore that these organisms should contain intracellular gas bubbles that have not yet been released into the environment. These gas bubbles were observed for the first time in 2012 by Swart and co-workers in Saccharomyces [1]. In this study, we investigated the conserved status of gas bubble formation in fungi, using the filamentous fungus, Rhizopus oryzae. To achieve this, R. oryzae was cultivated in fermentable and non-fermentable media and analysed using Light Microscopy (LM), Transmission Electron Microscopy (TEM) and Nano Scanning Auger Microscopy (NanoSAM). LM results indicated that gas bubbles were present in R. oryzae when cultivated in fermentable media, and this was confirmed by TEM and NanoSAM analysis. Gas bubbles were only present in small numbers due to respiration when cultivated in non-fermentable media. We concluded that gas bubble formation might be conserved in Fungi, although more research needs to be done to confirm this.

Many Bt crops are cultivated, but Bt maize stands out as the most widely grown Bt crop globally. Bt maize is maize (*Zea mays* L.) that has been genetically engineered to express the Cry1Ab gene from *Bacillus thuringiensis* (Bt) and produce an insecticidal toxin. These toxins are released into soil via root exudates, pollen, and plant residues and may affect the quality of soil and microbial communities. The study aimed to investigate the impact of Bt maize on soil microbial communities and enzymatic activities. Soil samples were collected from fields under irrigation and dryland conventional cultivation, where Bt maize had been planted at least for three consecutive years. Transgenic Bt maize expressing the Cry1Ab protein (event MON 810) and a near-isogenic non-Bt line were used for the study. Soil samples were analysed for enzyme activities that included acid phosphatase, β-glucosidase and urease. Rhizosphere communities were also studied using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). There were significant differences in β-glucosidase and acid phosphatase activities between Bt and non-Bt maize, while urease showed no significant differences.

Irrigated fields showed higher acid phosphatase and β-glucosidase activities in soils under non-Bt maize cultivation as compared to Bt maize. DGGE results showed no great variation amongst Bt and non-Bt maize banding profiles for bacterial communities under irrigation and dryland conditions. Fungal communities displayed a great variation of banding patterns between Bt and non-Bt maize under both these practices. Early findings suggest cultivation practices have an influence on soil enzymatic activities and microbial communities.
Contamination of foods by fungi and mycotoxins has been linked to various health and economic implications to both man and animals. This study was carried out to evaluate the incidence of fungal species and mycotoxins contaminating maize grains in the North West province of South Africa. A total of 100 maize samples were randomly collected from commercial and small-scale farmers across the province. Samples were investigated for fungal contamination using conventional and molecular methods to identify fungal species. Mycotoxin analysis was done using IAC, TLC, HPLC and ELISA. The percentage incidence of different genera isolated revealed the predominance of Fusarium (82%), Penicillium, (63%) and Aspergillus species (33%). Among the species, Fusarium verticilloides had the highest incidence of 70 and 76% in commercial and small-scale maize respectively, while P. digitatum had 56% total incidence and Aspergillus fumigatus (27%). Mycotoxin analysis revealed that FB₁ was the most contaminant mycotoxin in the small-scale and commercial samples with incident rate of 100 and 98.6% respectively. Aflatoxins contamination in samples occurred at incidences of 26.7% in small-scale samples and 25.0% in commercial samples. Furthermore, OTA had a high incident rate of 97.8% and 93.0% and ranged from 3.60-19.44 to 1.60-9.89 µg/kg respectively in small-scale and commercial maize samples. Zearalenone (ZEA) occurred in 50 and 55% of small-scale and commercial samples respectively. The results showed that maize from small-scale farmers may contribute to dietary exposure to mycotoxins. Farmers and consumers should be aware of the dangers of mycotoxin contamination of maize with resultant health risks.
Skin and soft tissue infections are a cause of high morbidity rates worldwide due to the emergence of bacteria resistant to conventional antibiotics. Medicinal plants like *Platycarpha glomerata* which have been reported traditionally to be useful in treating these infections might be the key to finding alternative treatment options. Different solvent crude extracts of *P. glomerata* (leaf and rhizome) at 10 mg/ml were screened against bacteria that are commonly associated with skin and soft tissue infections respectively using the agar well diffusion method. The MIC and the MBC values of the extracts were determined using the broth micro-dilution assay, while the chequer board method was used to assess the extracts’ interactions with ciprofloxacin. The membrane damaging potentials of the extracts against selected bacteria was determined by measuring the % cytosolic lactate dehydrogenase released in comparison to 3% Triton X-100. *P. glomerata* extracts had zones of inhibition ranging from 9.67±2.52 to 15.67±0.58 mm. The MIC values of *P. glomerata* extracts ranged from 5-10 mg/ml, with bactericidal activity being observed against *S. aureus* (ATCC 25925) only by all the extracts except the DCM leaf extracts. The results on membrane damage showed a low percentage release of cytosolic LDH. The extracts of the DCM (rhizome and leaf), acetone (rhizome and leaf) with ciprofloxacin showed synergistic interactions. The results of this study therefore, show that *P. glomerata* is a potential source of antibacterial compounds for the treatment of skin and soft tissue bacterial infection.
S15.O5 – DEVELOPMENT OF PCR ASSAY FOR ACCURATE IDENTIFICATION OF MACERGENS IN EXPORTABLE VEGETABLES OF SOUTH AFRICA

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Testing of vegetables to guarantee security and quality is the foundation of a sustainable quality certification program. Current method commonly utilized for detection of macergens is tedious and time consuming. This results in low productivity and economic losses of the vegetable availability in South Africa. Thus, there is a need for rapid detection of these macergens before importation and exportation of the vegetables in and out of the country. This study utilized Primer3Plus Platform to construct primers for fast identification of macergens. Four primers designed were then synthesized and used in quick detection of the macergen in exportable vegetable samples from South Africa. The nucleotide sequences of the detected macergens were deposited in the GenBank for scientific usage. In spite of the heterogeneity of these macergens phylogenetic analyses revealed their similarities and evolutionary trends.
Humankind’s demands for variety in fermented foods are constantly increasing much more rapidly than the number of yeast applications available to cope with current and impending challenges. Up to date, the baker’s yeast, *Saccharomyces cerevisiae* remains by far the most extensively exploited yeast in food, beer, wine and fermented products. The trending healthy lifestyles demand not only for conventional but new products necessitates the search for novel extreme and/or complex traits beyond this yeast. An immense natural yeast biodiversity on Earth at our disposal, non-conventional or non-*Saccharomyces* yeasts, present a vast untapped potential for industrial applications. However, even if an immense biodiversity exist, not many types of yeast isolates have the natural phenotypic traits that are directly transferrable for industrial applications. Here we report a long-term experiment, entailing the ‘replaying’ of the evolutionary ‘tape’ by mimicking the primordial environment, in which yeast and bacteria co-existed during the appearance of early angiosperms. A fierce competition for available sugars probably arose between yeasts and bacteria and other microbes. We therefore designed a serial dilution transfer method in batch cultures in the presence of initial excess glucose under aerobic conditions modified by sequentially introducing bacteria to “probe” yeasts. 18 yeasts covering a wide phylogenetic background spanning 400 million years of decent were evolved for at most 1200 generations. Variants generated from this work exhibited a 2-fold increased fermentative lifestyle and stress resistance such as thermotolerance and resistance to ethanol. Associated large-scale chromosomal rearrangements, point mutations and gene duplications were behind the “improved” traits. Our results highlight the novelty of a cross-kingdom competition to develop yeast genetic variants with new phenotypes for industrial applications.
The exhaustion of fossil fuels is driving research for renewable sources of energy. Waste sugarcane leaves (WSCL) as agricultural residues are potential feedstocks for biofuel production. This work focuses on the optimization of xylose and glucose release from WSCL subjected to different acid-based pretreatments. These fermentable sugars (FS) were subsequently used to optimize the physico-chemical parameters for hydrogen production and a techno-economic analysis for a large scale plant was carried out. To optimize the release of FS, three models were developed using HCl, H₂SO₄ and HNO₃ pre-treatments subjected to variable acid concentration, temperature, solid to liquid ratio and heating time. The HCl-based model showed highest coefficients of determination (R²) of 0.80 and 0.86 for xylose and glucose with optimal yields of 78g/L and 11.48g/L respectively. For hydrogen production optimization, the input parameters consisted of sugar concentration, inoculum concentration and Hydraulic Retention Time (HRT). The model gave an R² value of 0.91 with optimum setpoints of 14g/L FS, 32% (v/v) inoculum concentration and 62h HRT. Scale up studies using these optima in a 13L bioreactor showed a peak hydrogen fraction and a cumulative volume of 38% and 1873ml respectively. A techno-economic analysis was subsequently carried for a large scale biohydrogen production plant. The simulated plant has a capacity of 55 x 10⁴ kg sugarcane leaves/year and produces 4 x 10⁶ L H₂/year for a unit production cost of $0.96/L. These findings highlight the feasibility of utilizing waste sugarcane leaves for the production of FS, the subsequent production of hydrogen and an industrial scale up.
Rapid industrial development has led to environmental pollution with heavy metals which pose a great risk to the ecosystem. Heavy metal pollution is a worldwide problem affecting both developed and developing countries of the world. Bacteria have developed various mechanisms in tolerating heavy metals in the environment and thus making them suitable candidates compared to chemical methods in bioremediation of heavy metal contaminated soil and tailings.

A total of 56 heavy metal resistant bacteria were isolated from tailings and soil samples collected from three abandoned gold mine tailings sites in Krugersdorp, South Africa using Luria Berthani agar supplemented with heavy metals (aluminium, cadmium, chromium, lead, zinc, nickel, cobalt and copper (Al, Cd, Cr, Pb, Zn, Ni, Co and Cu). The most promising of the isolates are KSDC and KFTI which showed multiple and high metal resistant (5mM Nickel, 7mM Pb, 9mM Zn, 10mM Cr and 5mM Cd ) and were morphologically and physiologically characterized. The results obtained from this study showed that these isolates have the potential to be used in bioremediation of heavy metal contaminated environments.

Key words: Heavy metal, environmental pollution, bioremediation, mine tailings, soil.
The growing prevalence of infections associated with poor drinking water quality and the emergence of new pathogenic organisms that are resistant to current antimicrobial therapies, together with a decrease in the number of compounds entering the market for treatment of these diseases presents a major challenge. It is therefore imperative to search for new, efficient and safe antimicrobials with novel mechanisms of action and that are active against a broad range of pathogens.

*Streptomyces pharetrae* CZA14T used in this study has the potential to produce a number of bioactive compounds when conditions for production are optimised. Fermentation conditions for maximum antimicrobial production by CZA14T were scaled-up from 0.5 L, 1 L and 2 L baffled flasks to fermentation in a 3 L air-lift bioreactor and 5 L continuous stirred tank reactor (CSTR). Solvent extractions were performed on both the supernatant and mycelium to test for extra- and intra-cellular production. Extracts were partially purified using silica gel column chromatography and ammonium sulphate precipitation. Crude extracts were analysed using Thin Layer Chromatography (TLC) and the inhibitory efficacy of the crude and partially purified extracts determined using filter disk diffusion and bioautography assays against a broad range of DRWH isolates.

Crude extracts prepared from the different fermentation processes showed bands with similar Rf values on TLC plates when visualised under UV light. A broad bioactivity range was observed against all 14 DRWH isolates tested in this study. Further purification of these compounds and identification of pure compounds to structure level will be the focus of future studies.
Vaccination remains one of the most feasible ways to prevent losses due to lactococcosis, but knowledge of the genetic diversity and surface antigenicity of the bacteria isolated from the diseased fish is essential for development of effective vaccines. Lactic acid bacteria from three different genera (*Lactococcus*, *Weissella* and *Carnobacterium*) were identified through sequencing of the 16S rDNA gene to be the main bacterial species responsible for lactococcosis of farmed rainbow trout (*Onchorhynchus mykiss*) in Lesotho and the Mpumalanga Province of South Africa. A total of 21 *Lactococcus* strains (20 *L. garvieae* and 1 *Lactococcus sp.*), two *Weissella sp.* and three *Carnobacterium maltaromaticum* strains were assessed for their cross reactivity using seven rabbit produced antibodies (six anti-*L. garvieae* and 1 anti-*Weissella sp.* antibodies) using an Enzyme-Linked Immunosorbant Assay (ELISA). The morphological characteristics (colony morphology, Gram-stain and hemolytic activity on blood agar), phenotypic characteristics (growth at different temperature, pH and salinity) and API 50CH strips were used for assessment of metabolic activity. We were able to demonstrate that more than one *L. garvieae* serotype exists and none of the *C. maltaromaticum* strains (KM409658, KM409660 and KM409659) showed cross reactivity. Conversely, both *Weissella* strains tested in this study (KM409656, KM409657) cross-reacted with one of the anti-*L. garvieae* antibodies from strains KM409680, suggesting shared surface antigens. The information derived from this study will assist in the selection of strains to be used for development of effective vaccines against lactococcosis.
A novel 25 kDa L-2-haloacid dehalogenase (L-2-DhlB) from a recently isolated *Ancylobacter aquaticus* strain UV5 indigenous to contaminated site in South Africa is reported here with its gene sequence. The enzyme was purified to 22.1-fold increase in specific activity of 72.9 U/mg protein when the organism was grown in medium supplemented with 5 mM 1,2-dichloroethane (1,2-DCA). L-2-DhlB was optimally active at pH 9.0 and 37°C with poor stability at 50°C, retaining 50% of its activity after 30 min, but inactivated rapidly at 60 °C. L-2-DhlB catalyzed monochloroacetate (MCA) with Km and Vmax values of 0.47 mM and 2.4µM/min, respectively. L-2-DhlB exhibited the $k_{cat}$ value of 4.8/min. Expression of about 100% relative activity of L-2-DhlB on the substrate L-2-monochloropropionate (L-2-MCPA) as compared to 5% on D-2-monochloropropionate (D-2-MCPA) suggested that L-2-DhlB belongs to the family of L-2-haloacid dehalogenases. ES-mass spectroscopy and bioinformatics tools resulted in 693bp ORF sequence corresponding to 230 amino acid protein. NCBI-BLAST of L-2-DhlB resulted in the detection of a putative conserved domain of hypothetical haloacid dehalogenase (HAD)-like superfamily and subfamily IA.
Thermostable enzymes are of special industrial interest and have considerable importance in the multibillion dollar biotechnology industry due to their robustness and suitability to harsh processing conditions. The thermophilic compost-dwelling fungus *Thermomyces lanuginosus* is an attractive source of various thermostable enzymes. The anti-nutrient phytate hydrolysing enzyme phytase is presently the top animal feed-enzyme in the global enzyme market, while chitinases are known for their morphogenetic and developmental roles in plants, fungi and insects. In this study, we demonstrate the multifarious applications of phytase and chitinases from *T. lanuginosus*.

Phytase improved the growth characteristics of bean plants as indicated by a 2.06- and 1.41-fold increase in plant dry weight and shoot-length with a simultaneous improvement in inorganic phosphate content by 5.46-fold. Addition of phytase reduced the viscosity and phytate content of amadumbe flour, resulting in 1.62-fold improvement in ethanol production. Phytase also improved the nutritional characteristics of the local non-alcoholic beverage, *Mageu* by improving its Ca$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ content with a concomitant 12 h reduction in fermentation time. Similarly, multiple chitinases were investigated for several applications. Chit1 was better suited for production of chitobiose, while Chit2 can be exploited for its antifungal properties. Interestingly, a truncated chitinase (Chit 1') from *T. lanuginosus* showed 70% mortality against the second instar larvae of *Eldana saccharina*, the common sugarcane stem-borer of KwaZulu-Natal, and delayed pupation of other stem-borers such as *Sesamia calamistris* and *Chilo partellus*. Overall, thermostable phytase and chitinases from *T. lanuginosus* could be useful for a myriad of key biotechnological applications.
Grape producers and wine makers in South Africa are currently affected by various challenges, which include anti-alcohol lobbies, climate change, over-production in some vintages, and the lack of transformation and empowerment in certain sectors of the industry. An umbrella project aims to provide an alternate outlet for wine grapes by producing a Balsamic-styled vinegar. Due to lower production costs than wine, this can be an opportunity for small business entrepreneurs. The present study aims at understanding the contribution of a consortium of non-Saccharomyces yeast and acetic acid bacteria during the production of Balsamic-styled vinegar. Fermentation-acetification trials inoculated with a defined mixed culture of yeast and bacteria are being monitored using analytical chemistry, classic microbiology and molecular biology methods. These include substrate consumption, product formation, and microbial growth kinetics and polymerase chain reaction for population dynamics. The results so far have shown that the yeasts and bacteria involved do not survive equally well, and some may not have any contribution at all. These results suggest that some of the microorganisms can be eliminated during the process to decrease production cost. The final goal would be to optimize the fermentation-acetification conditions using response surface methodology.
Increased biodiesel production has increased the availability of the by-product, glycerol, since it is produced at 10% (w/w). Glycerol is an environmental problem as disposal is expensive and not environmentally friendly. Due to the high abundance the cost of glycerol has plummeted which has resulted in a drive towards its use as the sole carbon source for microorganisms in industrial processes. Not all microorganisms optimally use glycerol as a carbon source and glycerol from biodiesel production contains contaminants such as methanol, salts and water. It is the high salt contamination in particular that hinders most microorganisms’ growth with the exception of *Streptomyces albulus*. Little was known about the genetics of the gram-positive bacteria called *S. albulus*, which produces the value product ε-poly-L-lysine (PL). PL is an antimicrobial agent and is used as a food preservative in countries such as USA and Japan. We published the first draft genome of *S. albulus* in 2013. The genome is 9.43 Mb in size with a G+C content of 72.2% and contains 9,177 protein-coding sequences. Here we also report that *S. albulus* is a halotolerant microorganism capable of utilising biodiesel derived crude glycerol as its sole carbon source. The draft *Streptomyces albulus* genome reveals the biosynthetic pathway genes required for the production of the compatible solutes ectoine, hydroxy ectoine and tehalose, all osmolyte protectants, thereby supporting growth within this suboptimal medium. Transcriptomic analysis of *S. albulus* has revealed several differentially expressed genes during the normal growth phase of the bacteria. More importantly it has spread light onto the gene expression of PL synthesis and it indicates that the mechanism of control lies on the enzymatic side and not the genetic side.
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The first author of this article and colleagues earlier reported the role played by rhizosphere bacterial antagonists, Bacillus subtilis PFMRI and Paenibacillus macerans PF9, as bioprotectant and plant growth promoting rhizobacteria (PGPRB). Since, the strains were isolated from the rhizosphere, a diverse and complex environment, we hypothesized that the strains that are able to survive in such competitive environment could be of potential source of multiple hydrolytic enzymes with ability to biodegrade macromolecules, as well. Accordingly, the strains were characterized biochemically and physiologically. Besides, a number of hydrolytic enzymes of the two strains were extracted using carboxymethyl cellulose (CMC), pectin, starch and birchwood xylan as substrates and comparatively analysed for their respective catalytic activities and enzyme kinetics. Consequently, a number of hydrolytic enzymes, namely, cellulase, pectinase, xylanase and amylase, with important physiochemical properties were extracted from B. subtilis PFMRI and P. macerans PF9. Accordingly, the optimal pH and temperature of enzymes from the former strain were found to vary from 5.0 to 9.0 and 50°C to 65°C, while for the ones from the latter strain varied from 5.5 to 9.0 and 40°C to 55°C, respectively. Whereas the maximum velocity (Vmax), the amount substrate needed to reach half Vmax (Km) and time needed to reach half Vmax under optimal condition (Km) for enzymes from the former strain varied from 1128.64 to 13241.86 µmol.min⁻¹.L⁻¹, 1.81 to 205.1 mM, 0.19 to 8.04 min, while for the ones from the latter strain values varied from 3565.10 to 15366.68 µmol.min⁻¹.L⁻¹, 6.1 to 114.6 mM, 1.54 to 2.86 min, respectively. The present study is first of its kind in reporting the bioprospecting of hydrolytic enzymes from bacterial antagonists for biodegradation of macromolecules. Accordingly, a number of hydrolytic enzymes stable at elevated temperatures and pH extremes as well as with higher catalytic dynamics and substrate affinity were identified. Besides, we do anticipate that the new parameter, Kmt, would help us know the time limit of an enzymatic reaction and manipulate the reaction as needed. Thus, it is anticipated that such enzymes would be of potential role in the white industry. Yet, further study should be conducted to reverse engineer and work on heterologous expression of such enzymes so as to manipulate them for improved physiochemical as well as kinetic traits.

Key words: Bioprospecting, biochemical, bacterial antagonists, enzyme, macromolecule, biodegradation, enzyme kinetics.
We previously reported on a 3-hydroxy fatty acid (3-OH 9:0) molecule that is released into the surrounding environment of Cryptococcus neoformans UOFS Y-1378. Towards this end, we sought to determine if this molecule may exert an antimicrobial effect on Pseudomonas (P.) aeruginosa. Thus in vitro susceptibility tests were performed to assess the response of P. aeruginosa towards 3-OH 9:0. In addition, we determined the mode of action employed by 3-OH 9:0 in killing Pseudomonas cells. Pseudomonas cells were revealed to have a dose-dependent response profile i.e. 12% growth reduction at 0.2 mM and 32% growth reduction at 1 mM, when compared to non-treated cells. Corollary, we also observed a dose-dependent reduction in pyocyanin production viz. 82% reduction at 0.2 mM and 93% reduction at 1 mM. In our study, 3-hydroxy fatty acid-treatment of cells resulted in the disruption of membrane function – possibly through incorporation of this saturated fatty acid into the membrane bilayer, which in turn, may have led to a more rigid bilayer. The latter is supported by the inability of 3-OH 9:0-treated cells to release significant ($p < 0.05$) amounts of adenylate kinase into the supernatant. Furthermore, the disruption of membrane also led to significant ($p < 0.05$) accumulation of reactive oxygen species (ROS) in treated cells. These data assigns, for the first time, a function to these molecules as antimicrobial agents. Therefore, it is reasonable to conceive that these molecules would allow Cr. neoformans UOFS Y-1378 to appropriate an environmental advantage over other microbes in nature.

**Key words:** 3-Hydroxy fatty acids (3-OH 9:0); Antimicrobial; Cryptococcus; Pseudomonas.
Fairy Circles (FCs) are mysterious circular areas devoid of vegetation which are endemic to the Namib Desert of southwestern Africa. These barren patches have a life span of 24-75 years, after which surrounding grass repopulates the barren patch. Various hypotheses regarding FC origin have been proposed, including termite/ant foraging, subterranean gas seeps, plant self-organising growth patterning, and subtle variations in soil physicochemistry. However, the majority of these hypotheses remain poorly supported. Here, we investigate the hypothesis that microorganisms are involved in FC formation and/or maintenance. With the use of MiSeq amplicon sequencing and meta-proteomics, we assess microbial diversity and whether microbial pathogenesis is responsible for the formation of these structures. The direct detection of phytopathogenic proteins (toxins) or identification of metabolic pathway/s that lead to the synthesis of these compounds would be suggestive of a pathogenic mechanism. Ten surface (0-5cm) and ten deep (30cm) soil samples from the central zones of FCs and from vegetated external control soils were analysed. Sequencing of bacterial and archaeal 16S rRNA genes and fungal ITS regions were used to determine whether microbial communities within FC soils were distinct from those in surrounding vegetated control soils. Furthermore, LC-MS/MS analysis of soil protein extracts is used to compare the exoproteomes of FC and control soils, and to identify protein signatures which are unique to FC samples.
S20.O4 – SAMPLING AND SCREENING OF HYDROCARBON DEGRADING BACILLUS SPECIES.

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Hydrocarbon contamination due to petroleum products is a widespread problem, both locally and internationally. Biocontrol agents that can aid in remediation of soils, waters and other environments are required to provide sustainable solutions to this problem. The Bioprocess Development Group at CSIR has embarked on a national screening programme targeted at the identification and screening process of hydrocarbon degrading Bacilli.

Samples have been collected from several sites across South Africa. Cultures from these samples have been grown in growth medium containing diesel to enrich for target species and to induce sporulation. The stringent isolation protocol used selects for spore-forming Bacilli. A rapid colorimetric screening method employing the dye 2,6 DCPIP, was used to identify Bacilli with hydrocarbon degrading potential. A colour change from blue to colourless indicates the presence of hydrocarbon breakdown products.

A number of putative hydrocarbon degrading Bacilli were identified using the rapid screening assay. A specific isolate, morphologically identified as a Bacillus cereus, resulted in a significant discoloration of the dye after a 10 minute reaction time. Preliminary growth studies indicated good results, but the upstream process requires further development.
Salmonella, a major water and food borne pathogen, pose health risks in developed and developing countries. The management of infections caused by Salmonella spp. during outbreaks or forecasting of contamination of aquatic resources largely depends on sensitive, robust and rapid detection. Particles absorbs bacteria and sediment samples proves to be a better representative of microbial load than aquatic ones. Despite the advances and use of a highly efficient quantification and detection techniques like qPCR, challenges in bacterial detection in complex environmental samples remain due to presence of PCR inhibitors. The relatively, new droplet digital PCR (ddPCR) is a direct quantitative method which relinquishes the necessity for calibration/standard curve and is highly tolerant to PCR inhibitors. There is only limited information on the application of ddPCR to detect Salmonella in environmental samples and sediments. In this study, qPCR and the ddPCR were compared for the quantitation of Salmonella targeting the ttr gene in sediments collected from the Palmeit river close to Quarry road informal settlements, Durban. The site within the informal settlements exhibits Salmonella in the range of 255±37 and 818±30 Salmonella/gram (p≤ 0.0001) in qPCR and ddPCR respectively. The significantly higher numbers encountered with ddPCR might be due to the partitioning strategy where the PCR reaction is split into 20,000 droplets, ideally each droplet contains 1 or less copies of targeted DNA and efficiently reducing the effect of PCR inhibitors. In summary, the ddPCR was demonstrated to be a promising technology for the quantitation of Salmonella in sediment samples.
Filamentous bacteria are well documented as causative agents of bulking and foaming in the biological wastewater treatment process. These filamentous bacteria are however closely associated with other non-filamentous organism forming a micro-niche. Among these specific epiphytic bacteria attach to filaments in the consortium of organisms that make up the floc. Neither the eco-physiological role of the epiphytes nor the nature of the interaction between the epiphytic bacteria and the filament hosts they colonize is well understood and in need of in-depth investigations. The focus of this presentation is on the interaction between the epiphytic bacteria and the filament host.

Samples from the activated sludge treatment has been repeatedly collected from several wastewater treatment plants in KwaZulu Natal. Extensive investigations has been performed with SEM and TEM electron microscopy, Polarized Light Microscopy with Congo Red staining, and Thioflavin T staining to document the interaction. SEM was used to document the morphology of both the filament host and their epiphytes counterparts with the focus on the interface/point of contact between the two, while the main focus of the TEM investigations with the higher magnification aimed to document the ultra-structure features of two organisms relating to the interaction.

The interaction of the perpendicular attachment partly seems to be governed by the physiological status of the filaments. The attachment further seem to trigger a response in the filaments with distinct internal visible structures at the attachment sites. It is postulated that these structures most likely are amyloid fibrils. Amyloid fibrils may play an overarching role in different types of attachments and has earlier been noted to play a significant role in biofilm formation in activated sludge. They also play a medical role in degenerative diseases such as Alzheimer’s and Diabetes. Further studies aims to define the eco-physiological role of amyloid fibrils in filamentous bacteria, based on their observed presence at interaction sites in this study. This will also relate to additional findings where selectivity within the species of epiphytes attaching to the selected filaments has been noted.

Basic understanding of the eco-physiological interaction between two closely associated species or groups may have significant impact in the future understanding of wastewater treatment processes and broaden existing knowledge on population dynamics.

**Keywords**: Activated sludge; filamentous bacteria; epiphytic bacteria; TEM; SEM; EPS layer, FISH, Amyloid proteins
The antibiogram of Acinetobacter isolates from freshwater and soil samples in Alice and Fort Beaufort towns in Nkonkobe Municipality, South Africa were assessed for their extended beta-lactamase (ESBLs) spectrum. Eighty-six Acinetobacter isolates were obtained from the 50 samples of soil and 50 samples of water (25 in each location) analysed. The resistance of the Acinetobacter isolates ranged between 30-100% against penicillin G, ceftriaxone, nitrofurantoin, erythromycin and amoxicillin-clavulanic acid, while 9% showed intermediate response to minocycline, and 10% were resistant to oxytetracycline. Both Tet B and Tet 39 were detected in 66.7 % of the tetracycline resistant Acinetobacter isolates and in 44.4 % of the intermediately tetracycline resistant Acinetobacter isolates. An observation of 9.3% phenotypic expression of ESBLs was made while 3.5% were carrying the blaCTX-M-1 gene; all of which were susceptible to the fluoroquinolone. The multiple antibiotic resistance (MAR) index of > 0.2 indicates that the isolates emerged from high-risk sources, in line with conventional standards. Commensal Acinetobacter spp. in the environment have proven to be one of the reservoirs for antibiotic resistance genes.
S21.O4 – INVESTIGATING THE QUORUM SENSING INHIBITORY AND ANTI-VIRULENCE POTENTIAL OF SEAWEED-ASSOCIATED BACTERIA.

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Currently the primary mode of treatment for bacterial infections remains the prescription of antimicrobial agents. However, with the increasing frequency of antimicrobial-resistant, pathogenic bacteria, alternative treatments need to be sought. One potential strategy is the use of quorum sensing inhibition (QSI) which targets virulence of bacterial pathogens without causing cell death, avoiding an evolutionary move towards resistance. Bacteria from eight seaweeds were screened for their QSI ability against pathogenic microorganisms. Approximately 100 isolates underwent initial QSI activity screening using sandwich assays, with two Chromobacterium violaceum biosensor strains. Of these, 33 isolates were able to inhibit only short chain acyl homoserine lactones (AHLs), 13 degraded long chain AHLs and 16 were able to degrade both. Thirty isolates with QSI abilities and unique colony morphologies were selected for ethyl acetate extractions following submerged flask fermentations. Extracts were employed in overlay and quantitative violacein inhibition using C. violaceum, as well as pyocyanin inhibition assays using Pseudomonas aeruginosa. A reduction in violacein production without cell death was observed with six extracts using the overlay assay, indicating putative QSI activity of extracts. This inhibition was then quantified using the violacein inhibition assay. When tested against P. aeruginosa, selected extracts such as MAB2, MAB4 and AB3-SW6 were capable of QSI without causing cell death, with some isolates causing ≥ 94% inhibition of pyocyanin production, following exposure to ≤ 1 mg/ml of crude extracts. Based on the results obtained, metabolites from seaweed-associated bacteria may provide viable alternatives to antimicrobial therapy in the form of QSI.
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Global climate change is predicted to affect marine ecosystems, due to increases in atmospheric CO$_2$. The Southern Ocean (SO) is a pivotal ecosystem in terms of its role in regulating the Earth’s climate. However, we know very little about the correlation between microbial diversity and functional processes in this ecosystem and, more specifically, how depth may influence this relationship. To reduce this knowledge gap, we applied Illumina based amplicon pyrosequencing and Shotgun metagenomic analysis to assess microbial diversity and functional capacity in the SO. Ocean water samples from the Crossroads (CR) transect were retrieved on the R/V S.A. Agulhas II. Samples were collected at pre-selected depths: (a) deep (~ 10 m above seafloor), (b) middle (O$_2$ minimum) and (c) surface (fluorescence maximum). We found high taxonomic richness in surface and deep samples, with generally low numbers for middle samples, corresponding to O$_2$ minimum zones. ANOSIM analysis revealed marked differences between the three sample types dominated by marine bacterial (Proteobacteria, Firmicutes and Bacteroidetes) with smaller fraction of eukaryotes (Opisthokonta and Viridiplantae) and archaeal (Euryarchaeota) lineages. The results of functional annotation mirrored the taxonomic data with surface and deep samples showing the highest proportions of functional genes. Our data showed the first evidence of biogeochemical capacity (C, N, S), with a large proportion showing homology to those of Proteobacteria (Rhizobiales), and Cyanobacteria (genus Synechoccus). Taken together, our results unveil crucial functional cues for biogeochemical cycling in the SO and provide a solid baseline for understanding future perturbations and consequent impacts on biogeochemical cycling.
The most common acid rock drainage (ARD) remediation strategies result in neutralization of the waste water, precipitation of the majority of heavy metals and the reduction of the sulphate load. However, the residual sulphate concentration still exceeds the discharge specifications. Biological sulphate reduction processes represent an alternative and potentially more sustainable option to reduce the high sulphate load associated with ARD. These biological sulphate reduction processes make use of a consortium of anaerobic bacteria to catalyse the reduction of sulphate. The end products of sulphate reducing bacteria metabolism are sulphide and bicarbonate alkalinity, which from an ARD treatment perspective can be used for metal precipitation and neutralization, respectively. A number of sulphate reducing enrichment cultures have been developed, on simple and complex electron donors, from environmental samples and are being maintained within several reactor configurations, including a continuously stirred tank bioreactor, an anaerobic up-flow sludge blanket reactor and a linear flow channel reactor. These processes are being operated under increasingly stringent conditions and biological sulphate reduction performance monitored daily. To date there has been relatively little research conducted on the dynamic microbial ecology of sulphate reducing bacterial systems, however, the advent of culture-independent molecular techniques has facilitated a more comprehensive assessment of the microbial ecology of complex systems. This investigation makes use of a range of molecular tools, including real-time PCR primers and fluorescent in situ hybridization probes, to investigate sulphate reducing bacterial communities. The ability to evaluate the structure of these mixed microbial community is expected to impact on the design and operation of associated bioprocesses.
S22.O3 - CRYPTOCOCCAL 3-HYDROXY FATTY ACIDS PROTECT CELLS AGAINST AMOEBA PHAGOCYTOSIS


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We previously reported on a 3-hydroxy fatty acid that is secreted via cryptococcal capsular protuberances - possibly to promote pathogenesis and survival. Thus, we investigated the role of this molecule in mediating the fate of Cryptococcus (C.) neoformans and the related species C. gattii when predated upon by amoebae. We show that this molecule protects cells against the phagocytic effects of amoebae. C. neoformans (which produces 3-hydroxy fatty acids) was less sensitive towards amoebae compared to C. gattii (which does not) and addition of 3-OH fatty acids to C. gattii culture media, causes this strain to become more resistant to amoeba predation. Conversely, addition of aspirin (a 3-hydroxy fatty acid inhibitor) to C. neoformans culture media made cells more susceptible to amoebae. Our data suggest that this molecule is secreted at a high enough concentration to effect intracellular signalling within amoeba, which in turn, promotes fungal survival.

Key words: 3-Hydroxy fatty acids; Amoeba; Cryptococcus; Phagocytosis; Protection.
Source water for drinking water production is becoming a scarce resource in South Africa. Industrial, agricultural, urban and mining developments along our important rivers have negative impacts on the available raw water. Studies have shown that water purification plants in South Africa may not always produce the quality and quantity of drinking water they are designed for. The Hazards Assessment Critical Control Point (HACCP) concept is a preventative approach taken from the food industry where it ensures food safety. It has been successfully applied in the water industry and focuses on analysing specific critical control points within the water purification process. Statistical process control is a technique which can be implemented concurrently with the HACCP concept. It is a method which can be used to monitor and control a process through statistical analysis which would ideally improve the process. The use of Artificial Neural Networks (ANNs) in water quality is an area of increasing interest. ANN combined with Evolutionary Algorithm is useful to establish an improved monitoring model for water purification processes. This study aims to improve monitoring of water treatment processes with statistical process control by using HACCP as basis. Turbidity is a quick and cost-effective way of analysing the efficiency of a water purification plant. However, some of the other parameters could also provide important control data for the purification process. A preliminary model using historic data was prepared by applying ANN software. From this model other informative role players, other than turbidity, could be identified in the purification process. In this study three different monitoring models with various rule sets will be established and compared.
The effects of antimitochondrial drugs on Cryptococcus neoformans and Cryptococcus gattii.

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The emergence of HIV has led to increased host vulnerability to Cryptococcus neoformans and Cryptococcus gattii infections. Further to the point, the usage of fluconazole and amphotericin B in clinical settings to treat these infections is often limited by drug resistance development and undesired side effects. Therefore, there is a need to find “new“ drugs to better manage these infections more so in developing countries. Towards this end, we explored the repurposing of aspirin and ibuprofen as potential antimicrobials. In vitro susceptibility tests were performed to determine the response of Cryptococcus neoformans and Cryptococcus gattii towards aspirin and ibuprofen, and ibuprofen in combined therapy with amphotericin B and fluconazole. Additionally we sought to determine the mode of action of aspirin and ibuprofen. The studied fungal strains revealed a dose-dependent response profile towards aspirin and ibuprofen, with ibuprofen displaying greater activity. Subsequently, ibuprofen was paired with amphotericin B and fluconazole and was shown to enhance the effectiveness of tested conventional drugs. We also show that the two tested anti-inflammatory drugs kill cells through macromolecule damage following the induction of a stress-signalling pathway (high osmolarity glycerol pathway). The observed in vitro synergism implies fluconazole and amphotericin B can be added at low concentrations where clinically they could not exert any negative physiological outcomes. The major limitation in providing appropriate medical treatment is the cost of drugs, which is a pertinent issue in developing countries. Therefore, the therapeutic benefits of these anti-inflammatory drugs, which are relatively cheap, should now be considered.
A concerted action of α-galactosidases, β-mannanases and β-mannosidases is required to degrade the galactomannan in biofuel feedstocks such as sugarcane bagasse and Douglas-fir into galactose and mannose. However, the polysaccharides in these feedstocks are not readily available to the hydrolytic enzymes, and this necessitates pre-treatment to be conducted on the feedstock to increase polysaccharide accessibility. This process then leads to the formation of pre-treatment by-products, such as sugar and lignin degradation products, which can negatively affect the performance of these (mannanolytic) enzymes. In this study, five sugar degradation products, five lignin derivatives and four liquors from biomass feedstocks pre-treated by various technologies, were evaluated for their inhibitory effects on mannanolytic enzymes (α-galactosidase, Aga27A; β-mannanase, Man26A and β-mannosidase, Man5A). The organic acids significantly activated Aga27A activity, while moderately inhibiting Man5A and Man26A activities, respectively. Compared to the organic acids and furans, lignin derivatives elicited the greatest inhibitory effect on the mannanolytic enzymes. Gallic acid was the most inhibitory amongst the lignin derivatives, with potent inhibition of Aga27A and Man5A, but moderate inhibitory effects on Man26A. Lignin derivative inhibition appeared to be as a result of protein-phenolic complexation, leading to protein precipitating out of solution. The functional groups on the phenolic lignin derivatives appeared to be directly related to the ability of the phenolic to interfere with enzyme activity, with the phenolic containing the highest hydroxyl group content exhibiting the greatest inhibition. Using sugarcane bagasse, it was demonstrated that various pre-treatment technologies render different pre-treatment soluble by-products which interact in various ways with the mannanolytic enzymes. Using sugarcane bagasse and Douglas-fir, it was also demonstrated that different types of biomass (i.e. different plant species) release different by-products that interact with the mannanolytic enzymes in a diverse manner even when the biomass is pre-treated using the same technology (e.g. steam explosion). Enzyme inhibition by pre-treatment by-products can be alleviated through the removal of these compounds prior to enzymatic hydrolysis to maximize enzyme activity.
A recently isolated indigenous marine cyanobacterium, *Cyanothece* sp. was studied as a potential source of phycocyanin, a blue-coloured phycobiliprotein which has been used as a fluorescent probe and analytical reagent, as well as a natural dye in food and cosmetics. This study investigated the effects of several physicochemical parameters which included light, temperature, pH of the medium and NaCl concentration on the enhancement of phycocyanin biosynthesis and biomass yield. Increasing the temperature from 20 to 35°C and NaCl concentration 0.5-2.05M led to a significant increase (p>0.005) of 0.5 and 1.3g/l biomass respectively. Although the light intensity of 125 μmol m⁻².s⁻¹ favoured biomass production (3.3g.l⁻¹), a 26% decrease in phycocyanin content was observed. The initial pH of 10, light intensity of 100 μmol m⁻² s⁻¹, temperature of 35°C, and 2.05 M NaCl were found most suitable for the phycocyanin and biomass production. The maximum phycocyanin yield of 1.2 g.g⁻¹ dry cell weight was obtained. Phycocyanin biosynthesis is also critically influenced by the media components, thus standard BG11 medium was modified to enhance the phycocyanin production. Fractional factorial screening helped select NaNO₃, MgSO₄·4H₂O and minor nutrient stock has critical components that were subsequently optimised by central composite design using response surface methodology. The optimised media consisting of 0.12 g.l⁻¹ of MgSO₄, 1.8g.l⁻¹ of NaNO₃, and 11.25ml.l⁻¹ of minor nutrients resulted in a 3.5 fold increase in phycocyanin (5.85g.l⁻¹). This research revealed that this particular *Cyanothece* sp. shows great potential as a reliable source of phycocyanin.
Carbon dioxide (CO$_2$) and ethanol are by-products of alcoholic fermentation, which are excreted into the environment. Carbon dioxide is therefore expected to be present inside the cytoplasm of yeasts prior to eventual release. Previous studies [1] indicated that gas bubbles cannot be formed in the cytoplasm of cells; however Swart and co-workers [2] discovered gas bubbles in the Crabtree positive yeast, *Saccharomyces*. Consequently this study investigates the conserved status of gas bubble formation in Crabtree negative and strictly respiring yeasts. Yeasts that are Crabtree positive, Crabtree negative and strictly respiring were grown on fermentable and non-fermentable media and analysed with various microscopic techniques to determine the gas bubble status. Light microscopy indicated that Crabtree positive yeasts contained a large number of gas bubbles compared to Crabtree negative and strictly respiring yeasts. Results were verified with transmission electron microscopy and nano scanning Auger microscopy. The phenomenon of gas bubble formation seems to be conserved in yeasts; however the number of gas bubbles formed is affected by the mode of CO$_2$ production, i.e. fermentation, respiration or both.

**Keywords:** Fermentation; Gas bubbles

**References:**


Bacillus subtilis strain D014, is used in the production of biological products used for bioremediation, which ultimately enhance water quality. Therefore it is important to develop processes to efficiently produce this organism to meet cost and functionality barriers for market uptake. The bioremediation capability of the organism was previously demonstrated using synthetic waste water. The organism grew well on synthetic waste water and the percentage improvement in the removal rates when compared to the control for typical waste water pollutants such as ammonia, phosphates and chemical oxygen demand (COD) were 22.31, 15.72 and 20.39%, respectively. Enzyme assays further confirmed that isolate D014 was positive for the enzymes amylase, protease and cellulase.

During development of the fermentation process, variations to the base recipe were evaluated in 10 L Biostat C Bioreactors. A study was performed investigating different concentrations of the key nutrient source in the media, by reducing the recipe concentration of the nutrient by 20, 40, and 60% respectively. Key responses such as cell concentration, glucose concentration and sporulation efficiency were measured. The primary data was then modelled to calculate key performance indicators such as productivity, yield on protein (YPP) and yield on oxygen (YPO) which are of importance in commercial manufacture. Trend analysis indicated that there was a 3.89 and 4.87% reduction in productivity and sporulation efficiency, respectively, when the nutrient source was reduced by 40%. The measured parameters compared well to the base case. There was also an improvement of 34.31 and 1.86% in YPP and YPO, respectively. This recipe was favourable due to the significantly higher yield on protein and the improved control of foaming during operation. This data formed the basis for a validation experiment to statistically quantify the techno-economic performance and robustness of the process change. Initial studies were performed and triplicate fermentation batches resulted in a CV of less than 25% for the final cell concentration and productivity. This study formed a critical basis to improve the efficiency of the production process for the D014 organism, which enables its use in bioremediation applications for industrial and domestic waste water treatment.
Both wild, farm and domestic animals can serve as active reservoirs for the dissemination of multidrug resistant *Escherichia coli*. As of 2015, data regarding the ubiquity and virulence of such indicator bacteria within South Africa is limited. The antimicrobial resistance profiles of *Escherichia coli* isolates obtained from animals were established. Faecal samples were collected from indigenous herbivores such as zebra and giraffe and domestic and farm pigs from KwaZulu-Natal. Total and faecal coliforms and *E. coli* were quantified in faeces using a Most Probable Number (MPN) procedure and *E. coli* strains isolated from all samples used for further analysis. 150 randomly selected *E. coli* isolates from the various samples were characterized regarding their antibiotic resistance profiles according to EUCAST, using twelve selected antibiotics representing seven antibiotic classes. The MPN values for *E. coli* in zebra, wildebeest and giraffe faeces were 3.45, 4.73 and 4.96 log MPN/g respectively while the values for domestic and farm pig faeces were established as 5.11 and 5.15 log MPN/g. Of the 150 *E. coli* isolates analysed, approximately 21% were susceptible to all antibiotics tested. Isolates showing phenotypic resistance to antibiotics belonging to three or more classes were specified as multidrug resistant (MDR). While 43% of *E. coli* isolates from zebra were MDR, for wildebeest and giraffe this amounted to 20% and 3% respectively. Between 13% and 27% of *E. coli* isolates pig faeces were categorized as MDR. However, farm pig faeces was the only sample giving rise to an *E. coli* isolate resistant against fluoroquinolones.
The use of antimicrobial agents in aquaculture has significantly reduced options for treating fish diseases, due to the emergence of antimicrobial-resistant fish and opportunistic human pathogens. Research is now focused on seaweed as a prime resource in the search for microorganisms which demonstrate novel bioactivities. The antimicrobial and anti-biofilm potential of 96 seaweed-associated-actinomycetes (SWA) from eight South African seaweed, was thus investigated. Isolates were tested for their antimicrobial activity utilizing primary (cross streak assay) and secondary screening (agar well diffusion assay) against bacterial fish pathogens and the minimum inhibitory concentration determined. Bacterial isolates were screened by PCR and sequencing for the presence of polyketide and non-ribosomal synthetases genes. Extracts capable of inhibiting initial attachment and mature biofilm were identified using concentrations ranging from 1-10 mg/ml using microtiter plate assays. Primary screening against aquaculture pathogens indicated that 92% of isolates displayed activity against *Aeromonas salmonicida*, 51% against *Edwardsiella tarda*, 16% against *Vibrio parahaemolyticus*, 12% against *Salmonella arizonae*, 11% against *Yersinia ruckeri* and 4% against *Aeromonas hydrophila*. Following ethyl acetate extraction of 20 isolates, only MAB24 demonstrated bacteriostatic and bactericidal effects against all aquaculture indicator strains. Diverse combinations of polyketide and non-ribosomal synthetases genes were identified. Inhibition of initial adhesion was observed with ~70% of actinomycetes extracts and 50% demonstrated dispersion activity against mature biofilm in a dose-dependent manner without affecting growth. Seaweed-associated actinomycetes could be used as a potential source for the isolation of bioactive metabolites to combat biofilm production and the associated antimicrobial resistance of aquaculture pathogens.
The objective of this study was to determine the distribution and prevalence of superantigen (SAg) genes in Staphylococcus species recovered from bovine intramammary infections (IMIs) and close human contacts in the dairy environment. One hundred and fifty eight Staphylococcus aureus (146 bovine and 12 human) and 197 coagulase-negative staphylococci (CNS) (102 bovine and 95 human) isolates were screened for 19 SAg genes using a combination of five multiplex-PCR assays. No SAg genes were detected in the bovine or human CNS isolates evaluated in this investigation. Only 26.7% (39/146) S. aureus isolates of bovine origin tested positive for one or more SAg genes. The genes most frequently detected were sec (26%) and sell (26%) followed by seg (6.8%), sei (6.8%), seln (6.8%), selu (6.8%) and tst1 (6.8%). Only three SAg genotypes were detected amongst the bovine isolates. Amongst the S. aureus isolates of human origin 83.3% (10/12) tested positive for one or more SAg genes. Compared to the bovine isolates, eight SAg genotypes were detected. None of the bovine or human S. aureus isolates tested positive for the seb, sed, see, seh or selp genes. The relatively low occurrence of SAg genes amongst staphylococci implicated in bovine IMIs would suggest that the risk posed to close contact workers and public health, through the introduction of bacteria into the milk supply chain, is minimal. Due to the propensity for the dissemination of SAg-encoding mobile genetic elements amongst compatible bacteria residing in the same ecological niche, the possibility of a shift in the toxigenic potential of these bacterial populations may occur, warranting the need for continued monitoring.
Soy-daddawa is a fermented soybean condiment used in the preparation of traditional meals in Nigeria. Understanding the bacterial community dynamics during spontaneous fermentation of soy-daddawa is vital for a controlled fermentation process. The present study investigated the bacterial community dynamics during soy-daddawa fermentation by using both culture-dependent and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) techniques. Analyses of the pH, total titratable acidity (TTA), total viable count of bacteria and bacterial diversity were done at 24 h intervals during a 72-hour fermentation period. The isolates and PCR-DGGE bands were characterized based on 16S rRNA gene sequencing. Sequences obtained were clustered into operational taxonomic units (OTUs) at 97% sequence similarity and given taxonomic assignments. PCR-DGGE band profile was subjected to a hierarchical cluster analysis following densitometric analysis of DGGE image. The pH ranged from 7.01 to 8.19 while TTA ranged from 0.08 to 0.26 mg. lactic acid/g. TVC increased steadily with fermentation time and ranged from 3.9 to 10.61 log. CFU/g. A total of 58 isolates were obtained, which clustered into seven OTUs. Four OTUs were obtained from excised DGGE bands sequences. Taxonomic identification revealed that bacteria in soy-daddawa belonged to the genera Bacillus, Enterobacter, Enterococcus and Staphylococcus, with Bacillus sp. being dominant throughout fermentation. Amongst identified isolates were medically significant bacterial species such as Bacillus anthracis, Enterococcus casseflavus and Enterobacter hormaechii. These results emphasise the need for starter culture utilisation in guaranteeing the safety of the condiment and as well, offers a platform for a further starter culture screening and selection studies.
S24.05 - EVALUATING THE ACCURACY OF VIALBLE BACILLUS SPORE ENUMERATION

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Being able to effectively quantify microbial spore number and spore viability is of critical importance in assessing the quality of spore based products. Current protocols use microscopic spore counts and conventional viable plate count assays to quantify spore number and viability. Although these techniques are well established, they prove problematic for enumeration of highly concentrated Bacillus spore suspensions because of spore clumping, which results in an underestimation of viable spores. This study aimed to quantify the influence of spore concentration on clumping tendency and the subsequent effect on the accuracy of viable spore enumeration. Alternative methods for more accurate and rapid enumeration of viable spores were explored. A commercial strain of Bacillus cereus was used as the model organism for this study. Viability experiments using highly concentrated spore feed solutions (~1E+10 spores.ml⁻¹) concluded that only 19 ± 2% of spores where recovered using the viable cell counting procedure. Subsequent germination enhancement treatments increased viable spore recovery to 58 ± 7% of the total spores. Strategies to reduce spore clumping through dilution, mechanical and chemical dispersion resulted in further improvements. A 40 – 60% improvement in spore enumeration was achieved using these strategies. Preliminary data supports our hypothesis that spore clumping is the main cause of under-quantification of viable spores in highly concentrated spore solutions. The next phase of the study will focus on the application of new methods such as viability staining and flow cytometry, towards developing a rapid and accurate method for quantification of Bacillus spores.
Foot-and-mouth disease virus (FMDV) is a RNA virus, which causes a highly contagious economically important zoonotic disease, affecting mainly cloven-hoofed animals. FMDV is divided into seven serotypes (A, O, C, Asia 1, Southern African territories (SAT) 1, SAT2, and SAT3) each further divided into several topotypes relating to their geographic distribution. FMDV manifests sensitivity to acid and high temperatures, which is thought to be important for the release of RNA during cell entry. However, the stability of the virion, as exemplified by the 146S particle, has been associated with decreased induction of protective immune response following vaccination. In an attempt to genetically engineer vaccine seed viruses with improved capsid stability, we investigated the inherent stability of contemporary SAT1 viruses from southern Africa. Using a novel fluorescent thermal stability assays we have compared the disassembly of virions and inactivated 146S particles of SAT1 viruses at low and high pH, range of temperatures and various ionic strengths. Results indicated that when sucrose density gradient purified SAT1 viruses was exposed to increasing temperatures in increments of 0.5ºC for 10 seconds, an inherent seven degree difference in dissociation between the most stable and least stable virus was observed. A similar general stability trend was also observed when viruses was treated both with either pH buffer ranges or various ionic strengths. Even though FMDV, especially the SAT types, exhibit large intra- and inter-serotype genetic variability, the multiple and repetitive intersubunit interactions appear to be conserved within a serotype and even across serotypes.
Eleven bacterial strains were isolated from soil samples collected from mine tailings. The bacteria were checked for tolerance against heavy metals (Cr, Ni, and Cd). All the strains showed multiple tolerances against heavy metals but most promising results were given by strain BCr3, BCd33 and BNi11 that were tolerant to 15 mM of Cr$^{6+}$, 7.5 mM of Cd$^{2+}$ and 10 mM of Ni$^{2+}$ respectively. The effect of heavy metals on bacterial growth was tested together with their ability to grow in different pH, NaCl and temperature values. Bacterial strains grew well over a wide range of pH (6.5 – 8.5). The optimum temperature for maximum growth was between 35–37$^\circ$C and no significant change in bacterial growth was observed in presence of 2% NaCl. Bacterial strains BCr3, BCd33 and BNi11 showed high bioaccumulation ability of Cr (68.7%), Cd (72.4%) and Ni (69.8%) respectively. All bacterial isolates were identified by 16S rDNA gene sequencing. Analysis of plasmid content revealed that all bacterial isolates contained a single plasmid. Further, polymerase chain reaction was used to screen all bacterial isolates for the presence metal tolerant genes on both plasmid and chromosomal genomes.

**Keywords**: bioaccumulation, heavy metals, heavy metal tolerant bacteria, metal tolerant genes